

How to tackle global calamity

The Pugwash organization prefaces this year's statement on arms control with a declaration on the threat of global catastrophe by other means. It should now apply its traditional methods to this new field.

It is a canard that the Pugwash organization consists of people who are frankly left-wingers ('liberals' in the United States?) except when their political inclinations are so buried by innocence that they are naive. So much is clear from the composition of the meeting on the Black Sea two weeks ago (see *Nature* 335, 110; 1988). For several years, the annual Pugwash meeting has been a way of rubbing shoulders with retired generals and senior civil servants apparently relishing the experience of being able to speak out more openly than when they were in office. There is also usually a generous sprinkling of people who are far from soft on nuclear weapons in particular or military defence in general, but who appear to attend from a belief that those who would abolish nuclear weapons will be able to make a better case, and a less distracting one, if they know what their opponents say — and who, in any case, enjoy the discussions. And then there is always a strong (not necessarily large) delegation from the Soviet Union which reflects its contemporary government's view, together with a band of influential and independently minded people from Eastern Europe. Card-carrying 'peace-niks', while never absent, are by no means the majority. That people of such variety and experience spend a week each year (often with smaller workshops in between) asking how arms control might be made more effective, far from being shameful, is something to be proud of.

So much should be kept clearly in mind by those who read the statement on this year's meeting put out last week by the Pugwash council, and in particular the declaration "Ensuring the survival of civilization" with which the usual statement is prefaced. That is not a way of saying that the statement should be scorned. On the contrary, it is right to assert that there are other dangers than nuclear weapons (environmental destruction and the poverty of the nations said to be 'developing', but which often seem to be slipping backwards); avoiding nuclear war by means of arms control would be a hollow boast if the vestigial ice caps were then allowed to melt. It is also right to say that there is a 'web' of international problems, some environmental (ozone, for example) and some social (population growth), which threaten everybody's peace of mind. The obvious defects of the declaration are twofold. First, by seeking to mention every hazard, Pugwash fails to give the ordinary person a sense of where to start. Second, in striking contrast with the measured language in which the Pugwash council acknowledges the opportunities and difficulties facing arms controllers, the declaration on survival minimizes the difficulties by describing them as if they were simple consequences of people's waywardness, even some people's malevolence.

Disaster

Take population growth, one of the causes of the "denial of basic needs for a growing majority of mankind", which, with "excessive production and profligate consumerism", is said to be "pushing the planet to disaster". In the spirit of the hard-headed consideration traditionally lavished on issues in arms control, two questions arise: what kinds of disaster? And what can be done to avoid them? Sadly, rapidly growing populations may, for that reason, further impoverish themselves, outstrip their

natural food supplies and be decimated by famine, disease or natural disasters: the toll taken by the floods in Bangladesh in the past two weeks is a vivid proof of that. Yet for all the tragedy of these events, they are not global but national disasters. People in more prosperous countries may organize airlifts, or spend their Sundays running through their city parks, but it is the people of Bangladesh who will have to live with the consequences in the decades ahead, during which there will be other floods. It is the same in the Sudan and in Ethiopia (where it is hard to tell to what degree natural calamity or government policy should be blamed). Not surprisingly, there seems to be an empirical rule that members of the fastest growing populations are most at risk of untimely death (although the risk is less than formerly, which is why the populations are growing).

Solutions

And the solutions? The traditional method, based on the example of the developed countries but also of many in South-East Asia, is that birth rates usually fall to match falling death rates, but lag by a generation or so. Whether this demographic transition will apply in all developing countries is another matter; family patterns are not all the same. So far, only China has been able to limit population growth by fiat, in a draconian example probably not widely applicable. In most other places, exhortation from outside, often regarded as unwarrantable interference, is commonplace, which is not to diminish the value of what people from inside and outside do to help spread information about contraceptive techniques and the like. It is true that general assistance from overseas, but especially medical care and nutrition for children, should help, but only in the long run. So it is important that those regarding population growth, as distinct from natural disasters (which certainly have wider side-effects) as the harbinger of catastrophe should be prepared to say how urgent they believe the problem to be: can it wait a generation or so, or must something be done sooner, in which case, what? No aid without population control, perhaps, which would be inhumane? Sadly, the declaration does not say.

Much the same is true of the other threats to human survival listed in the declaration — water pollution, deforestation, soil and species conservation, ozone depletion and the accumulation of carbon dioxide. It is true to assert that all these questions are part of a web, but it is not a strictly seamless web. This journal has been urging for some time that a start should be made on the greenhouse problem, not because the threat is imminent, but because it is a serious threat and one that could be irreversible (if the ice should melt). Socially and economically, this is a threat to compare with that of nuclear war, if less bizarre. By comparison, most other phenomena to which people are exposed may be judged less serious. So why not say that, or something like it, to guide the actions of the people who will read the declaration? By embracing all the causes of discontent its authors have been able to think of, they have made it ineffectual.

That is yet another reason why Pugwash should think hard before deciding to take up cudgels for this broader cause (there is no suggestion that traditional interests should be abandoned). It is, of course, correct to assert that security is no longer simply



a matter of military strength, but there is as yet no reason to believe that the proper management of the global environment will be free from difficulties of the kind that have plagued arms control over the decades — chauvinism, prevarication, fecklessness and downright selfishness. That is where the Pugwash experience could be invaluable. □

Everything is money

Finance ministers should not be surprised that their economies seem uncontrollable.

THE best part of a decade ago, the variety of economists called monetarists came into the ascendancy in several places with a simple and beguiling recipe for running the money affairs of modern states, themselves tired of a decade of inflation. The monetarist recipe is what the name implies: control the supply of money in people's pockets, they said, and you will regulate the economy. If, for example, the supply of money is constant but there are more goods for sale in the shops, prices will decline because one supply must requite the other (which is broadly speaking true, provided that the speed at which money circulates is not increased). On both sides of the Atlantic and, now, in Australasia as well, versions of this recipe have been successfully applied to beat back inflation without bringing economic growth to a dead stop. So why are monetarists not now hailed as protectors of the modern state, saviours of civilization everywhere? Because ordinary people, in their ordinary ways, turned out to be too smart.

The dilemma is illustrated by what has gone wrong in Britain and the United States. The British case is the simpler, involving an over-simple definition of what constitutes money. In 1979, the newly elected government imposed on itself (and its voters) the discipline of limiting the growth of a quantity called 'M3', essentially the sum of the total cash in people's pockets and instruments such as bank deposits they could quickly turn into cash. By three years ago, the target was manifestly too low and was abandoned on the grounds that, with the British economy in better shape than for decades, the fault must lie with the target, not economic policy. Having begun this year by selling pounds for dollars so as to prevent their value increasing, the monetary authorities are now probably on the opposite tack, buying pounds to sustain sterling because people fear that inflation is on the way back. What has gone wrong?

Over short periods of time, the cash in people's pockets and banks may accurately measure spending power, but not when timescales lengthen. Indeed, given time enough, people can turn virtually any asset into spending power, either by selling it or by using it as collateral for a loan from the bank. Even expectations (the prospect of a better job, the sudden illness of a wealthy aunt) are bankable. Ordinarily there are limits to the amount of spending power that can be generated like this, but the British have evaded them by playing a real-life version of the game 'Monopoly' with each others' houses, driving up prices with the help of tax-breaks on mortgages, making everybody feel wealthy and filling many pockets with the real stuff. The government is now trying to put out the fire by increasing interest rates, but it should be tackling the trouble at source.

In the United States, affairs are more complicated. The now outgoing administration first cut taxes, creating a sense of well-being, putting more money into pockets — and then borrowing it back to cover the federal deficit. The snag is that the assets, say Treasury bonds, acquired in return seem like wealth even though the government may not be ready to redeem them (and they are certainly bankable). So the United States has run up a trade deficit as well as a federal deficit because the government, like the British, did not appreciate that people who want to spend money will find ways of doing so, whatever the statistics say. The process has entailed the sale of a large slice of US industry to people from overseas. Soon, it will have to stop. □

Tricks to order

US regulators offer sensible guidelines for investigating fraud, but should keep their distance.

SCIENTIFIC misconduct as now defined (see page 195) is not new, only the likelihood that there will now be legislation on the subject in the United States. It has always been likely that the whiffs and puffs of laboratory scandal in the past few years would dangerously attract attention among the federal agencies and in the Congress. Now those chickens are coming home to roost. The Department of Health and Human Services (HHS) plans to take over from the National Institutes of Health the management of the investigation of alleged fraud in the scientific literature. No doubt mounting interest in the Congress has left it with little choice. But HHS appears not to appreciate how serious will be the implications of its proposals.

As HHS itself acknowledges, what is now called scientific fraud is not fraud in the ordinary sense. Most criminals convicted under this heading have deceived others for the sake of tangible gain, usually a pot of money. Although there have been some cases in which scientists have been accused of deception for the sake of monetary gain, the more effective exploitation of a patent, perhaps, the cases in the front of the mind of Congress have been aimed at other goals — a published paper, other marks of professional esteem and even professional promotion. The victims in these cases are the scientific community at large, which is cheated, close professional colleagues (who may be damaged by association), the institutions that employ the fabricators and plagiarists — and the people themselves, at least if they are found out. Technically the grant-making agencies are also deceived, which is why HHS enters the suit.

As they are, the regulations HHS proposes are sensible. It is a splendid idea that, if there is to be an investigation of misconduct, it should be quick. Delayed or prolonged inquiries help private and public relationships to fester. (Delay is also not always distinguishable from avoidance.) Institutions faced with the need to investigate allegations against one of their members should prudently call on outsiders to do what is bound to be a distasteful and thankless job. And so on. If HHS confines itself to overseeing these processes, not carrying them out itself, not much extra damage will be done. The danger is that Congress will push the agencies into a more inquisitorial role, or even assume such a role itself.

That would be disastrous. For what the world at large does not appreciate is that, except for the most flamboyant cases of fraud, there is no certain way of distinguishing between malevolence and honest error, which is as unavoidable in research as in any other profession. It is even forgivable, given the emotional attachment to his creation of the author of a piece of research or of a report thereon, that he should be reluctant to withdraw. If under the new regime or some other, people who make honest mistakes are to be prosecuted with the zeal that should attach to those who set out to cheat, that will sour the atmosphere of productive laboratories. But there can be no question that the only sure judges of a person's motives are his or her colleagues, the closer the better. For all their virtues in other fields, members of the Congress are not well-equipped for these sombre tasks, which works against the doctrine that the assessors should be independent.

The moral is that the Congress should stay away from the administration of these matters, contenting itself with an occasional retrospective inquiry into the way in which institutions look into cases brought to light. In any case, there are other cases more directly in the purview of Congress where miscalculation has cost taxpayers real money (the B-2 bomber development or, for that matter, the search for Z^0 and $W^\pm$ mesons at the Stanford Linear Accelerator Center). To pretend that science could be infallible is foolish, but to behave as if discovery can be regulated by judicial rules is downright wrong. □

James Watson to head NIH human genome project

- Congress wants independent advisory body
- International coordinating group set up

Washington

THE National Institutes of Health (NIH) will announce later this month that James Watson, the director of the Cold Spring Harbor Laboratory, will become associate director of NIH. Watson will head a newly established office that will coordinate NIH efforts to map and sequence complex genomes.

The genome office, part of the office of the director, will not distribute research grants, but will instead provide general policy direction, identify neglected



Watson — gets new projects off the ground.

research topics and target new technology. Watson has said that he also intends to encourage active investigations into the ethical issues raised by a genetic map of the human genome. Decisions about research grants will continue to fall to the National Institute of General Medical Science, which has a budget this year of just under the \$28 million in the Reagan administration's 1989 budget request.

Apart from his stature in the scientific community, Watson was thought the ideal choice for the job because of his ability to get new scientific projects off the ground. He is also popular with Congress which will ultimately have to pay for genome activities. Some wonder if, by making the transition from trusted outside adviser to a member of the federal bureaucracy, his relationship with Congress will change for the worse.

The decision to create a new associate director's position is a direct consequence of a meeting held last March at the behest of NIH director James Wyngaarden (see *Nature* 332, 99; 1988). Watson has been holding discussions with NIH since last spring over the possibility of taking the new position. He is expected to average only one day a week at Bethesda.

Although the NIH genome office has

not yet officially opened for business, NIH has been moving on the project. It is in the process of concluding a memorandum of understanding with the Department of Energy (DoE) over genome activities. The memorandum would formalize existing informal relationships, and would establish an interagency working group to identify areas for joint activities.

The extremely cordial relations between the two agencies is in part explained by their desire to forestall legislation now pending in Congress that would establish an independent panel to advise on genome activities. The legislation, originated by Senator Lawton Chiles (Democrat, Florida) and passed by the Senate, calls for a national advisory panel on the human genome consisting of the Secretary of Energy, the directors of NIH, the National Science Foundation and the National Library of Medicine as well as four representatives from private industry, four academic researchers, one bioethicist and one representative from charitable foundations. Although both NIH and DoE acknowledge the need for an outside advisory panel, both would apparently prefer to establish their own advisory mechanism.

The Chiles bill is now awaiting action in the House of Representatives. In addition to establishing a genome advisory panel, it also establishes panels for biotechnology and agricultural biotechnology. Three different committees were given jurisdiction to hold hearings on the bill. One, the Science Space and Technology Committee, has completed its work, and the Agriculture Committee is expected to finish this week. That leaves the powerful Committee on Energy and Commerce chaired by Representative John Dingell (Democrat, Michigan), which has yet to schedule action on the bill.

As the domestic debate over who should provide guidance for the genome project continues, an international group is forming to add its voice to the chorus. HUGO, the Human Genome Organization, is an extra-governmental group that met last week in Lausanne, Switzerland, to discuss coordination of international efforts on the project. The meeting, paid for by the Howard Hughes Medical Institute, had representatives from Europe, the United States, the Soviet Union and Japan. The group has established an executive committee that will be headed by Lennart Philipson, the director of the European Molecular Biology Laboratory.

Uranium restrictions accepted by India

Bangalore

THE Indian government seems willing to accept stringent conditions for the supply of enriched uranium by the Soviet Union, despite opposition within its own Department of Atomic Energy.

According to M.R. Srinivasan, chairman of the Atomic Energy Commission, a draft agreement for the supply of two Soviet 1,000-MW pressurized light-water reactors is now under consideration. The terms already agreed by India include Soviet supply of enriched uranium for the power plants and the return to the Soviet Union of all used fuel rods.

India refused to return US-supplied fuel rods used in the Tarapur nuclear power plant to the United States on the grounds that India had paid for the fuel. The United States would not accept this position because of fears that plutonium could be extracted from the rods and used to make nuclear weapons.

Within the department, agreeing to similar Soviet regulations is seen as a blow to India's stand. Protests are likely to follow which will also draw attention to the poor safety record and lack of diagnostic systems in Soviet nuclear systems.

Radhakrishna Rao

Britain wasting time

London

HIGH-LEVEL radioactive waste should be buried in deep repositories underground without further delay. That is one of the main conclusions of a report published last week by the House of Lords Select Committee on the European Communities. "No better solution is discernible and it is irresponsible to wait any longer for some hoped-for but unspecified alternative." To allay public fears, these repositories should be monitored over a period but must eventually be permanently sealed, says the committee. In Britain, high- and intermediate-level waste is at present stored where it is produced, pending a decision on disposal.

The committee is also satisfied that low-level waste can be satisfactorily disposed of in shallow repositories. All categories of waste can be suitably and permanently disposed of and decisions to do so should not be deferred to future generations, it says.

Christine McGourty

It has also established a finance committee that will look for funds to keep the group going. The Hughes Institute provided only enough to get the group started, and it has made no decision about future funding commitments.

Victor McKusick of Johns Hopkins University in Baltimore chaired the HUGO meeting. Joseph Palca

Japan's budget request up by 13 per cent on this year

Tokyo

SUPERCONDUCTORS, AIDS and international cooperation take top billing in the budget requests for fiscal year 1989 just submitted by Japan's science-related ministries and agencies. Alongside are requests for the backing to develop a large helical fusion device and to initiate national projects on neurocomputers, hypersonic transport and technology for digging super-deep tunnels.

The budget requests for all government organizations total more than ¥64 million million (about \$500,000 million), 13 per cent up on last year. The Ministry of Finance is expected to trim these requests to about ¥58 million million when the budget is finalized at the end of this year. But appropriations for science and technology are usually little changed in the end-of-year negotiations.

Perhaps the most original idea in this year's budget comes from the Ministry of International Trade and Industry (MITI). MITI wants to develop technology to tunnel deep under Japan's overcrowded cities. The project is a ploy to get around extortionate land prices. The idea is to tunnel deeper than 50 m and create gigantic caves in which factories and electric power generators will be installed. By going deeper than 50 m, the ministry hopes to reduce drastically the costs paid

Titan launches

Washington

A MODIFIED Titan 2 rocket was used by the US Air Force on 5 September to launch an undisclosed payload from Vandenberg Air Force Base in California. It was widely reported that the rocket placed four US Navy satellites, collectively known as White Cloud, into low polar orbit, from where they will track shipping and eavesdrop on electronic communications.

The launch came only three days after another Titan 2 launch from Cape Canaveral. But the Associated Press reported that the upper stage failed to ignite properly, and a satellite designed to monitor Soviet communications did not reach its intended geosynchronous orbit.

The two launches herald a large programme of non-shuttle launching for military purposes. A number of Titans have been converted from intercontinental ballistic missiles to satellite launchers, and new rockets are on the order books. Although shuttle launches are due to resume soon, with several places reserved for military payloads (*Nature* 335, 105; 1988), there is a backlog of such satellites waiting to be launched. David Lindley

for space to surface landowners whose ownership rights are limited to a depth of a few tens of metres. The ministry has requested ¥30 million to study the tunnelling technology and to look at the legalities of land ownership deep underground.

Several ministries want to boost funds for superconductor research. MITI has requested ¥4,480 million (\$33 million), ¥1,000 million more than for this fiscal year. Much of the increase will go to a larger contract for the International Superconductivity Technology Center set up earlier this year by private industry and MITI to develop the new high-temperature superconductors. There is also a large increase in outlays under the energy-saving 'Moonlight Project' to develop a 70,000-kW superconducting generator that will employ conventional nickel-titanium superconducting wire.

Similarly, the Science and Technology Agency is seeking an extra ¥1,000 million for research on the new high-temperature superconductors under its 'multi-core project' which involves various of the agency's research institutes; a total of ¥3,082 million is requested.

The Ministry of Education, Science and Culture will launch two new superconductor projects next year under its grant category 'priority areas of research' which support research by large groups of scientists with annual funds of a few million dollars. Hiroshi Hara of Chiba Institute of Technology will head a three-year project entitled 'new electronics based on high-temperature superconductors' while 'chemical design and processing of the new high-temperature oxide superconductors' will be led by Kazuo Fueki of the Science University of Tokyo.

The ministry of education's largest new request, however, is for a giant helical fusion device which will employ superconducting magnets and will be housed in a new national research institute to be built in Gifu Prefecture (see *Nature* 335, 104; 1988); ¥2,480 million is requested for research and development of the superconducting magnets, ¥572 million to begin construction of the new institute and ¥1,699 million for administration and running costs (excluding personnel costs).

The Ministry of Health and Welfare wants to increase outlay for the fight against AIDS by more than 70 per cent. The ministry has requested ¥1,383 million for research and development of anti-AIDS drugs and vaccines, ¥251 million for AIDS prevention and counselling measures, ¥178 million for running the ministry's new AIDS Research Center and AIDS Medical Information Center, set up earlier this year in Tokyo in the

National Institute of Health and National Medical Center respectively, and \$2.5 million for the World Health Organization's anti-AIDS programme.

The ministry is also seeking ¥236 million to start a project to isolate and characterize the virus responsible for hepatitis non-A non-B which is a major health hazard in Japan. Experiments on chimpanzees will be carried out in collaboration with US scientists at the Liberian Institute for Biomedical Research, which was established in Africa by the New York Blood Center. And the ministry has also applied for ¥550 million to invite about 60 foreign researchers to Japan, most of whom will be specialists in the study of this virus or AIDS.

Japan has been criticized for the imbalance in exchange of researchers with the West. All the science-related ministries and agencies have asked for extra funds to invite foreign researchers to Japan. The Science and Technology Agency has applied for ¥950 million for 130 postdoctoral fellows, up from 100 for this fiscal year, and the Ministry of Education has also applied for support for another 130 (again up from 100). But the new fellowship programmes, which began only in this fiscal year, are suffering from a lack of applicants, and it is uncertain whether the Ministry of Finance will provide funds for more.

The Human Frontiers Science Program seems set to get under way at last. MITI and the Science and Technology Agency have requested a total of ¥2,586 million (\$19 million) for a foundation in Europe that will distribute international grants for research on the brain and on molecular recognition and response. The scheme will begin in autumn of next year.

MITI hopes to begin several new projects next year. Twenty million yen is set aside for a study of neurocomputers. A group at Tokyo University and several electronics companies, including NEC and Fujitsu, are carrying out small-scale research but MITI is now thinking of a national project.

MITI also hopes to get research under way on engines and airframe materials for a hypersonic plane. The ministry has requested ¥30 million to initiate a new project on hypersonic propulsion systems under the ministry's category of 'large-scale' industrial projects, and another ¥159 million to study development of a '75-seat class' hypersonic private transport facility.

In addition, MITI also plans to establish a new research centre to develop ceramics and carbon composite materials resistant to ultra-high temperatures for application in aviation and space technology. The centre will form part of the recently reorganized New Energy Development Organization (see *Nature* 333, 4; 1988).

David Swinbanks

US proposes new rules for dealing with misconduct

Washington

THE US Department of Health and Human Services (HHS) has proposed new rules for dealing with and reporting possible misconduct involving research paid for by the Public Health Service (PHS). The department will also publish a preliminary announcement that it will seek comments on several strategies designed to prevent scientific misconduct.

In July 1986, the National Institutes of Health produced guidelines for dealing with scientific misconduct as part of a set of rules governing all contracts and grants. But these do not have the same legal weight as departmental rules. Earlier this year, PHS sought to publish its own rules, only to find its plans thwarted by the White House Office of Management and Budget (OMB). OMB is said to have felt that PHS's first attempt was too restrictive, and asked for a new strategy.

PHS responded with two documents. One, which will take effect after a 60-day comment period, deals with the particular responsibilities of institutions in coping with charges of misconduct. The new rule defines misconduct in science as "serious deviation" from accepted practices for conducting research, such as plagiarism, or falsification or fabrication of data. Failing to comply with federal requirements for conducting research would also qualify as misconduct.

The new rules would require grantee institutions to notify the appropriate funding agency in writing of any misconduct investigations. The timetable for completing investigations would be 60 days for an initial inquiry, and 120 days for a complete investigation, with extensions only when requested in writing. These time periods may change depending on the comments received publication of the rules.

The second document is an attempt to develop before-the-fact prevention strategies. PHS makes it clear that the term 'scientific misconduct' has been chosen over scientific fraud because of the common legal definition of fraud. Robert Charrow, HHS deputy council, speaking last week to a workshop on scientific misconduct sponsored by the Institute of Medicine, explained that for the government to prove it has been the victim of fraud, it would have to show that a researcher had misrepresented his work at the time he applied for a federal grant. Charrow argues that a researcher could represent his data accurately even though he knew it was in error, making it impossible for the government to prove it was defrauded.

PHS is also trying to determine which agency should investigate allegations of

misconduct. Institutions which investigate questionable practices by their own faculty members face difficulties that may be insurmountable. PHS is considering placing all such investigations in the hands of the HHS Inspector General's office. To encourage compliance with proper research practices, PHS is also looking into imposing sanctions against institutions convicted of inappropriate actions.

PHS is not alone in pursuing these issues. In Congress, the House of Representatives government operations subcommittee on human resources, chaired

by Ted Weiss (Democrat, New York), has been considering possible legislative changes to encourage compliance with proper scientific practices. Following the Institute of Medicine workshop last week, the National Academy of Sciences Committee on Science, Education and Public Policy will transmit recommendations for preventing misconduct to the National Institutes of Health later this year. The American Association of Universities is also coordinating an effort involving 10 other groups to develop a comprehensive framework for dealing with misconduct. This framework will be discussed at a workshop sponsored by the American Association for the Advancement of Science and the American Bar Association later this month.

Joseph Palca

International dispute at the BA

Oxford

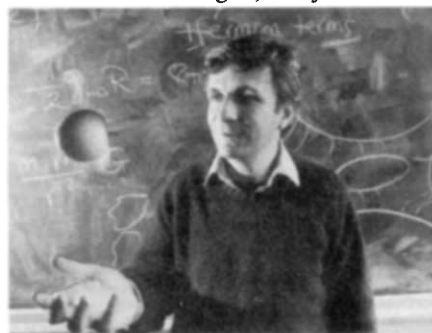
INTERNATIONAL collaboration in science is a good thing, agreed a group of eminent researchers from around the world in a debate at the annual meeting of the British Association for the Advancement of Science held in Oxford last week.

But the practical difficulties revealed in the course of the debate led even its chairman, Professor Sam Edwards of the University of Cambridge, to admit that his own policy on internationalism is "to encourage it and hope that it doesn't happen".

The discussion group included Dr Walter Massey, president of the BA's sister organization in the United States, Academician Rem Petrov, president of the Soviet Union's Society of Immunologists, and others from India, China, Africa and Europe. At first everyone heartily agreed that international cooperation is good for science, although the reasons given varied widely. Massey argued the wasteful expense of competition in big science projects. Petrov argued that collaboration gives valuable insight into other modes of thinking. But everyone seemed to agree that mechanisms to facilitate collaboration are necessary, and the speakers were applauded politely.

But the arrival of a rowdy bunch from the Institute of Physics late in the afternoon injected controversy into the proceedings; with several delegates trying to shout each other down, it was hard at times to keep track of the arguments. Someone suggested that researchers are forced into collaboration only when work in their local environment is unsuccessful. Many argued that policies for collaboration are unnecessary; they only increase the growing amount of scientific bureaucracy and can actually destroy collaboration at grassroots level. There were warnings of the build-up of a community of "scientific tourists", flying round the world with one motto — "have paper will travel".

And some international projects, with no single clearly defined aim, easily become "talking shops", it was claimed. As the chairman drew the debate to a close, six hours after it had begun, everyone hurried



Michael Green unifying the physical forces.

off, some no doubt counting the hours of research wasted in talking that day. Others, though hurried off to fight for a seat in the crowded lecture theatre where Professor Michael Green of Queen Mary College, London, was explaining the theory of superstrings.

Debate of a different kind raged in Oxford last week, with accusations flying around as to who should be responsible for reinjecting life into British science. Sir Walter Bodmer, this year's president of the association, criticized the government for the decreasing public support for science. But Mr Kenneth Baker, the Secretary of State for Education and Science, said the government was spending more than ever on basic research, and he lamented the lack of investment in science by British industry. He also questioned the value of conferences such as this one to resolve the issue. "The question is now widely recognized and discussed, but action must result or all this activity will have been a waste of time." And that action, he said, is not the government's responsibility; it can take place only within individual institutions and companies.

Christine McGourty

Neuroscience in Europe

Zürich

BIOLOGICAL research in Europe could be much more effective with more coordination between the research efforts of the various European nations. Neuroscience suffers particularly, because of its varied approaches. Some practical remedies were proposed at a special symposium at the European Neuroscience Association annual meeting, held here last week.

Attendance at the meeting is symptomatic of the problem — fewer than 2,000 people participated, out of an estimated 8,000 neuroscientists active in Europe. A disproportionately large number came from the smaller nations, and poorer participation from the larger countries means that the opportunity for making contacts cannot be fully exploited. Competition from specialist societies leads to further fragmentation that also damages interdisciplinary exchange.

Michel Cuénod, retiring president of the association and director of the Brain Research Institute in Zürich, identified the country-based structure of academic careers and research funding as a major problem: individuals tend to give first priority to national collaborations and meetings. But Europe has the advantage of large centres within easy reach of each other, which could be exploited, suggested Philippe Lazar (director-general, INSERM, Paris), by establishing supranational networks to concentrate effort on timely and important problems; these should involve regular short visits between laboratories rather than long-term exchanges. A cheap travel pass for scientists would remove one serious obstacle to frequent interchanges.

Funding such networks is more problematical: asking governments to contribute to European budgets may weaken national research funding. The main support for exchange visits at the moment comes from the European Science Foundation, whose neurosciences programmes are already oversubscribed.

An alternative, modelled on a scheme already operating in Switzerland, was proposed by H. van der Loos, professor of anatomy at the University of Lausanne. European-based drug companies should be asked to contribute to a supranational research fund, supervised by a committee of scientists who would determine priorities. The fund should be used for supporting basic research, with no industrial strings attached. Such a scheme might also help to solve a major problem facing the pharmaceutical industry: how to support basic science, the main source of industrially useful innovations, without bleeding the universities of their best talents.

Jennifer Altman

Superconductivity in UK suffers from apathy in industry

Cambridge

SUPERCONDUCTIVITY research in Britain is being held back because industry is unwilling to invest. Of £16 million available in public funds, only about £10 million has been spent. This is partly because superconductivity is officially classed as an advanced technology project and one of the funding criteria is that there must be three partners from industry or two from industry and one higher education institute. Sir Martin Wood, who chairs the national committee, said at a symposium on high-temperature superconductors at the University of Cambridge last week, that industry has been "somewhat slow" in coming forward. The attitude of industry, he said, is that if something is being done in Japan and the United States, then Britain will not be able to keep up and that if those countries are not involved then it is not worth doing.

Funds in Britain for high-temperature superconductivity are not available on the scale of funding elsewhere, said Wood, and if industry is to benefit then it must target specific areas where applications are seen to be likely. This contrasts with the situation in Japan where the fact that commercial benefit may be distant does not discourage investment in basic research on a large scale. And the situation is similar in the United States, where the attitude towards applications is illustrated by one researcher's comment at a conference in San Francisco last month: Dr Jan Evetts, a director of the new interdisciplinary research centre at Cambridge, reported the delegate as replying, when asked of the possible applications of a superconducting thin film, that you might be able to "scratch a message on it and send it through the post".

But in Britain, the funding arrangements require that industry is involved and industry wants to see applications on the horizon. Dr Derek Howarth of the Research and Technology Policy unit of the Department of Trade and Industry (DTI), speaking at the symposium, said that the present funding arrangements will probably change next year, possibly in an attempt to alert industry to market opportunities. Funds for high-temperature superconductivity will then be channelled through the government's LINK scheme. Whether the move will have the desired effect is debatable, but it may unlock some of the funds available, because a project will be required to have only one industrial partner. There will also have to be one partner from the science base involved. The switch will mean that most of the projects might change quite dramatically, said Howarth.

Funds for research in high-temperature superconductivity also come through the Science and Engineering Research Council (SERC), which spends £2 million a year, and through a joint arrangement between the council and the Ministry of Defence. Between £3 million and £5 million is available through this joint scheme but remains unspent. Peter Anderson of the ministry said this is because the scheme is not well known and because some researchers are hesitant about getting involved in defence-related research.

After finding funds, researchers in Britain are faced with another problem: how to keep up with developments in the rest of the world. Because of the expense, there is no comprehensive database in this country. Both the SERC and the DTI have examined bids for a database and rejected them on the grounds of cost. Now work is underway to establish links with existing databases in Europe.

Another obstacle to advances in this field is a shortage of postgraduate researchers. Short-term posts are not always attracting the required workers.

Researchers at the symposium also expressed concern that unless more money is spent on technology based on conventional superconductors, then when high-temperature superconductors are at an exploitable stage Britain will not have the technology to build on in order to reap the commercial benefits.

The new interdisciplinary research centre which hosted the symposium was set up this year with £5.3 million from the government. Thirty researchers are employed at the centre and there are posts available for another 30. As yet there are no contributions from industry, but the director, Dr Peter Duncumb, hopes eventually to have 20 per cent industrial sponsorship.

Christine McGourty

Drug testing at Seoul

London

A \$3-MILLION drug-testing laboratory is being set up for the Olympic Games in Seoul. The laboratory will operate 24 hours a day for two weeks and will test 2,000 urine samples for 4,000 banned substances. With the equipment, which has been supplied by Hewlett-Packard, chemists will test for five different classes of drugs: stimulants, such as amphetamines; narcotics, such as morphine; anabolic steroids, which accelerate muscle development; beta blockers, which reduce heart rate; and diuretics, which dilute urine to reduce the presence of other banned substances.

Christine McGourty

US scientists slow in grasping potential of supercomputers

Washington

DESPITE a late start, Japanese industry is buying more supercomputers than its US counterparts. And although US supercomputer manufacturers may still have a technological lead, US industry is not capitalizing on the advantages offered. Those themes lay behind a symposium on supercomputers organized in Washington last week by the National Research Council.

The precursors of today's fastest machines appeared in the early 1970s, and the first Cray-1 was delivered in 1976. But until recently, almost all the supercomputing capability of the United States resided in a few federal laboratories, and was largely reserved for classified defence work. Only a few academic researchers were persistent enough to obtain small allotments of supercomputing time.

As well as slowing the development of general applications and software for supercomputers, a more far-reaching effect, according to Jack Worlton of Los Alamos National Laboratory, was that for 15 years science and engineering students passed through universities ignorant of what supercomputers could do. Many of those students are now senior academic and industrial scientists, and are still slow to grasp the potential of modern computers.

Three years ago, the National Science Foundation (NSF) began its support of five national supercomputing centres. According to Larry Smarr, a pioneer of numerical astrophysics and now director of the National Center for Supercomputing Applications (NSAC) in Urbana, Illinois, a key aim is to educate scientists in the use of supercomputers. Smarr has also drawn industry into NSAC, attracting as much as \$1 million additional yearly funding from partners such as Eastman Kodak and Amoco.

Surprisingly, representatives from Kodak, Apple Computer and Abbott Laboratories, a pharmaceutical company, all agreed that it is generally easier to persuade management of the need for supercomputers than it is scientists. But by demonstrating how to simulate plastic injection moulding, heat generation in printed circuits or drug design by computational chemistry, NSAC scientists have been able to convince their colleagues in industry that supercomputers can speed and simplify product development.

To take full advantage of what supercomputers can offer, users must be able to tell machine designers what they need. Steve Chen, until recently chief designer at Cray and now president of his own company, argued that the simple pursuit

of computational speed was only a small part of supercomputer development. Compromises must be made, for example, between memory size and access speed, between speed of arithmetic operation and of data movement, between small numbers of large processors and vice versa; in all these choices, the type of computing job to be done influences the compromise to be made.

According to Smarr and Chen, Japanese technology trails American by perhaps

two years, perhaps a few months. US computer science holds a definite advantage in novel architecture (such as the massively parallel Connection Machine), but such innovations are a long way from widespread commercial applicability. The hardest problems facing supercomputer designers now are the relatively mundane constraints of materials science — electron conduction on nanosecond time-scales and heat dissipation, in particular — and these are areas in which the Japanese researchers have proven ability. A feeling emerged from the meeting that if the United States loses the lead it now possesses, much of the blame will rest on consumer indifference. **David Lindley**

Row over White House moves to ban fetal tissue research

Washington

A PRESIDENTIAL order that would have effectively banned the use of human fetal tissue for research or transplantation has been postponed, but not before causing considerable consternation among scientists and ethicists preparing to discuss such

soon as possible", and asking for comments by 9 September. As the letter was sent on Friday, and the following Monday was a national holiday, HHS had only three days to prepare its response. A copy of the order was leaked to the news media, and on Friday, 9 September the White House insisted that no change in policy was imminent.

The preemptory nature of the executive order was particularly upsetting to those planning to attend a meeting this week of the Human Fetal Tissue Transplantation Research Consultant Panel at the National Institutes of Health (NIH). The panel was formed last July at the request of Robert Windom, Assistant Secretary for Health, who placed a temporary ban on all federally funded research involving transplantation of human fetal tissue obtained from induced abortion. The panel is to discuss the scientific and ethical issues relating to such research and prepare its recommendations for Windom, who will then decide whether to lift the ban. Those associated with the meeting contended that there would be little point in holding it if the presidential order was implemented as written.

The timing of the order led many to conclude that the motivation was political rather than scientific or ethical. Lately, the Reagan Administration has shown an interest in opening up the debate over controversial topics relating to human life. In addition to this week's NIH meeting, the administration also this week published in the *Federal Register* a proposed new charter for the HHS Ethics Advisory Board. The board has not existed for eight years, and it must approve certain research projects, notably *in vitro* fertilization, before such projects can receive federal funds. These topics have already come up in the presidential campaign, and are likely to help divide the positions of the two candidates. **Joseph Palca**



"Apart from smearing Dukakis with, of course..."

issues in Bethesda next week at the request of the Reagan administration.

The order originated in the office of Gary Bauer, assistant to the president for policy development. In addition to banning fetal tissue research and removal of fetal organs not intended to benefit directly the fetus involved, it would also ban the use of such organs taken from a fetus recovered from an induced abortion. The edict would define life as beginning with fertilization, precluding research on certain contraceptives, such as the intra-uterine device, that interfere with implantation rather than fertilization.

In a letter dated 2 September, Bauer wrote to Otis Bowen, Secretary of Health and Human Services (HHS), informing him that the order would be put through the White House clearance process "as

Who leads the drugs league?

London

JAPAN is rising up the world league table of originators of successful drugs, according to a survey of 72 pharmaceutical companies and 500 products published this month by the London-based merchant bank Robert Fleming Securities Ltd. Of the world's top 50 best-selling drugs, seven originated in Japan, a country that would not have featured in the table at all a few years ago, says the report. The United States is at the top of the table with 20 of the top 50 drugs in 1987. Ten come from the United Kingdom, six from Switzerland, five from West Germany and one each from Sweden and Italy.

Of individual companies, Merck, of the United States, has the largest revenue, showing an ability to translate research into revenue greater than that of any other company. The UK company Glaxo lies fourth behind Ciba-Geigy of Switzerland and Hoechst of Germany, but is growing faster than they are; the report predicts that Glaxo will rise to second place behind Merck in 1988. A major factor in its rise is the "phenomenal success" of its anti-ulcer drug, Zantac, the world's top-selling drug since 1986, with sales of about \$1,500 million.

Drugs with markets growing strongly are those for the treatment of viral infections and cardiovascular disorders. Although antiviral drugs were almost non-existent a few years ago, the market grew by more than 60 per cent last year to \$500 million and is expected to increase four-fold over the next five years with new drugs for herpes and AIDS.

The market for cardiovascular drugs was worth about \$16,000 million last year and is expected to grow at an average annual rate of 15 per cent. Calcium antagonists and ACE inhibitors, drugs used to treat high blood pressure, are growing at the expense of beta-blockers. And cholesterol-reducing drugs and thrombolytics, which dissolve blood clots, have "particularly exciting prospects", says the report.

At the other end of the spectrum are drugs in decline, most notably Valium and Ativan, both tranquillizers that have been criticized for their addictive properties.

In general, innovation in the industry has been slow over the past two decades, says the report, reflected in the fact that of the best-selling drugs, 30 out of 50 have been at the top of the league since before 1980, and 10 of them since 1970. And when they decline it is often not a result of the emergence of innovative products but because of patent expiry and the subsequent emergence of similar competitors.

Christine McGourty

Computer software in India

New Delhi

BLESSED with a vast brain pool and with an eye on the growing market, India has launched an aggressive programme for producing computer software for export. The government has decided to set up four technology parks where engineers will write software for export via satellite.

The first software technology park at Pune, near Bombay should, by the end of this year, have 20 Indian companies with shops in the park sharing the satellite terminal directly linking their customers in US cities. In five years, software export from the park is expected to reach \$300 million. Three more parks are being established at Bubneswar, Bangalore and Chandigarh. Officials of the Department of Electronics (DOE) say that India could become the world's major software producer by the end of 1990.

The United States is the first target for export, followed by Japan and Europe. Potential US customers are being identified by the state-owned company CMC Limited in preparation for the establishment of an Indo-US software trade network. As part of the export drive, DOE has been organizing seminars abroad to project Indian software capabilities, and has set up a software development agency within the department.

India wants to keep its brightest graduates at home rather than seeing them emigrate to the United States and elsewhere. Software parks can keep them

gainfully employed while earning foreign currency for India.

The government has also welcomed foreign computer companies that recruit Indian staff. Two years ago, Texas Instruments (TI) of Dallas set up a subsidiary in Bangalore with a staff of 30 software specialists. An Earth station on top of its office building now sends the programme written by the Indians to TI's mainframes in Dallas via Bedford in England. With cheaper Indian labour, TI expects to recover its investment cost in another two years.

In another project, Indian engineers are generating financial software programmes for the US company Citibank at its subsidiary in a free-trade zone near Bombay airport. Hinditron Services of Bombay has been developing programmes for US companies Tetronix Inc., Digital Equipment Corporation and Hewlett-Packard. Japan has also spotted India's software potential, and PSI Systems in Bangalore develops software exclusively for Japanese computers.

India is also encouraging Indians living abroad to return home and invest their talent and savings in software parks. Two groups have so far responded and will invest \$5 million in each of the three proposed parks at Pune, Hyderabad and Nilgris (in Tamil Nadu) where the government will install Earth stations to link them with US computer companies.

K.S. Jayaraman

Air pollution takes its toll in the US

Washington

"ACROSS the United States, air pollution is contributing to the decline and ultimate death of forest trees and to widespread losses in crop yields." That is the conclusion of a study* by the World Resources Institute, and, according to the study's authors, the time has certainly come to take steps to mitigate this damage.

Although natural factors still account for most forest decline, airborne chemicals are playing an increasingly important role. Increased ozone is thought to be damaging pines in the San Bernardino National Forest near Los Angeles, as well as white pines in the eastern part of the United States. Red spruce in the east, yellow pines in the south-east and sugar maple in the north-east are also showing signs of damage. In addition to ozone, acidic pollutants in the air, as well as longer term damage from the deposition of these compounds in soil, are posing problems in forests.

Crop damage, primarily from ozone, has also reached significant levels. Using data from the National Crop Loss Assessment Network, the World Resources Institute

study estimates that ozone is causing more than \$3,000 million in losses for major crops. Estimates are that a 40 per cent reduction in ozone would all but eliminate the cash losses to agricultural crops.

To stem the damaging tide of pollution-caused destruction, the study calls for an integrated strategy involving decreased emissions from stationary and mobile sources, development of alternatives to burning fossil fuel, and maintaining a closer watch on the shifting picture of the interaction of pollutants with the biosphere. One problem in charting forest damage, says James J. MacKenzie, one of the report's authors, is that there are no national programmes to evaluate forest decline.

Ironically, after years of criticism, environmental groups are now seeing so-called Second Generation nuclear technologies as a potentially non-polluting alternative for power needs should renewable energy alternatives — wind turbines, solar cells, geothermal energy — fail to achieve their promise.

Joseph Palca

* Ill winds: airborne pollution's toll on trees and crops. World Resources Institute, Washington, 1988.

Troubled Soviet space effort

London

THE Soviet Union's current mission to Mars and its moon Phobos has been effectively halved. After 75 successful radio link-ups, monitoring trajectory parameters and the performance of on-board instruments, the link-up with the Phobos-1 probe scheduled for 2 September "failed to materialize", according to the TASS agency. Phobos-2, however, is working satisfactorily.

The failure seems to have been caused by a programming error when control of the flight was transferred from the regular control centre in the Crimea to one near Moscow. A flight controller, it seems, inserted an erroneous computer program which left the craft unable to receive commands for its solar panels, so that the batteries have become weak.

The Phobos probes were launched in July and will reach the vicinity of Mars early next year. Nineteen other countries are involved in the mission, either providing experiments and hardware, or as trackers. The US National Aeronautical and Space Administration, in particular, is scheduled to take part in a very-long-baseline interferometry tracing experiment, scheduled to start in October, which has now been put at risk. Fortunately, it is Phobos-2, not Phobos-1, that is scheduled to carry out the more spectacular parts of the mission, dropping the long-life autonomous station and the mobile lander — the hopper — which will be landed on the Martian moon, Phobos, and making the close (50-m) flyby and laser beam spectroscopy experiment on the moon's soil.

The suggestion that the failure was due to a programmer's error comes at a time when the shortcomings of the Soviet computer industry are under attack. The chairman of the Supreme Soviet's Science and Technology Commission, Igor Glebov, said in a recent radio interview that although the Soviet Union has 300,000 trained computer programmers, their productivity is low because they do not have the necessary equipment at their disposal.

Vera Rich

■ ACCORDING to the latest calculations from the European Space Agency, the Soviet surveillance satellite Cosmos 1900 will reenter the Earth's atmosphere on 9 October. The satellite carries a nuclear reactor and is likely to shower nuclear debris around its crash site. Where that will be is still unknown: at this stage ESA can predict only that it will be between 65°N and 65°S. If the satellite begins to tumble it will descend more rapidly and could reach the Earth as early as 20 September.

Alun Anderson

Next round in West Germany's biotechnology licensing struggle

Munich

BIOTECHNOLOGY companies and environmental activists are at odds in West Germany over the licensing of a biotechnology facility in Hannover. New regulations for production facilities using genetically engineered organisms took effect in West Germany on 1 September. But Invitron Corporation, the US company which is building the Hannover facility, received approval for its plans in August, before the new rules took effect.

Opponents of the plant say they will now use every legal means at their disposal to stop construction of the facility. Under the new regulations, included in the *Bundesimmissionsschutzgesetz* (federal emission protection act), public participation in the licensing procedure would have been required.

A spokesman for the Green party, Ruth Hammerbacher-Richter, objected to the non-public nature of the licensing procedure of Invitron and complained of a "completely insufficient review of the security aspects" of the new facility. She repeated the Greens' much-used argument that using cell cultures to produce pharmaceuticals is dangerous because retroviruses might grow in the cultures and endanger the population. The local Green party has already submitted an official protest against the licence.

More protests are expected in Marburg where Behring, a division of Hoechst, is

applying for permission for a facility using genetically engineered mouse cells to produce the anti-anaemia drug erythropoietin. Behring has resubmitted its application to allow for public discussion.

Marburg Green party member Marina Steindor said that her group would raise the aspect of the "social responsibility" of genetically engineered pharmaceuticals at the public hearings. She cited recent studies showing an unexpectedly high antibody response to human insulin produced using genetic engineering techniques.

For Invitron, a company based in St Louis that receives contracts from other companies to produce monoclonal antibodies and pharmaceuticals using cell cultures, the advantages of building a plant in West Germany outweigh the disadvantages so far. Rick Srigley, vice-president of Invitron, cited the strong reputation of the West German pharmaceutical industry and the large number of potential customers in the Hannover area as reasons for moving there. He seemed prepared to try to ride out the storm of protest. "It is up to the government", Srigley said, to support biotechnology in West Germany. If setting up a plant here "doesn't work out", he added, "the consequences will reach a lot further than Invitron".

The Bundestag (parliament) will deliberate later this year a 'gene law' covering the use of genetic engineering in research and industry. Steven Dickman

Accelerator for art's sake at the Louvre

Paris

THE world-famous Louvre art museum in Paris has just received its own Van der Graaf tandem ion accelerator. Although the accelerator would have been less out of place in the ultra-modern science museum at La Villette than underneath the Louvre's noble Carrousel gardens, ion-beam analysis is playing an increasing role in contemporary conservation and art historical research. The Louvre is the first major museum in the world to have its own dedicated accelerator.

Nicknamed AGLAE (Accélérateur Grand Louvre d'Analyse Élémentaire), after one of the three graces, the US-built accelerator and its shielded environment cost FF10 million (\$1.6 million), paid for by the Ministry of Culture and Communication. AGLAE will initially be used in proton-induced X-ray emission mode (PIXE) to enable X-ray spectrographic analysis of art objects within the French national collection, but five further modes are available for other analyses.

As the analysis is rapid, non-destructive



and can be carried out without leaving the tight museum security, even priceless and fragile works can be studied, providing a valuable database for art historians and for the detection of fraud.

Peter Coles

Controversy continues

SIR—We have followed with interest the debate resulting from the publication of Benveniste's paper¹ and the controversy over the reproducibility of results.

Biological systems are notoriously unpredictable because of our limited knowledge of their interactions, and repeated experiments are often required before a general trend can be discerned. It is surely unscientific to dismiss five years work on the basis of results obtained in a mere five days. Might not Maddox *et al.*² themselves be falling into the trap of insufficient data to eliminate sampling errors? It is, after all, much more difficult to prove a negative finding than a positive one. A similar criticism applies to the more recent reports since published^{3,4} which may also fall into the trap described by Gracely where observer expectation influences the results⁵.

The idea that homoeopathic principles "strike at the very basis of chemistry" and require that "the Law of Mass Action or Avogadro's number" be thrown away is illogical. It is often forgotten that homoeopathic remedies are frequently prescribed in material doses and that although the high dilutions, or high potencies, which are a later refinement, are of great clinical value⁶, they are not essential to the practice of homoeopathy, nor are they its basis.

Biology often presents us with apparent paradoxes which, with greater knowledge, are resolved. Not many years ago, the world of immunology was split by the controversy as to whether immunity was humoral or cell mediated. In the event, it turned out that both factors were important in the development of the immune reaction and the controversy ceased to exist. Perhaps sophisticated developments in quantum physics will similarly resolve the controversy over the nature of homoeopathic remedies.

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1. Davenas E. *et al.* *Nature* 333, 816 (1988).

2. Maddox J. *et al.* *Nature* 334, 287 (1988).

3. Seagrave J.C. *Nature* 334, 559 (1988).

4. Bonini, S. *et al.* *Nature* 334, 559 (1988).

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6. Gibson S.L.M. & Gibson R.G. *Homoeopathy for Everyone*, (Penguin, London, 1987).

SIR—The scientific world will reject the findings of Jacques Benveniste and for good reasons, although the argument for a biological effect at high dilution is unlikely to die. More interesting than the debate over the quality of these particular experiments are the reasons why the physical demonstration of such phenomena should have such an allure. James Randi, prob-

ably unconsciously, puts his finger on the answer when he describes the reaction of such claims as akin to that ensuing if he were to say, "I keep a unicorn in my back yard". The point, however, is not our incredulity at such a statement but the fact that we might wish it were true, and for reasons that have nothing to do with the rational mind but reflect a profound human need.

These 'high-dilution' experiments and much of homoeopathy evoke a similar response with their notions of alchemy. Their quasi-scientific explanations conceal an effect which relies upon engendering a sense of the wondrous and even the mystical. We wish to 'believe the unbelievable', to be over-awed and uncomprehending. Of course, the myths and legends served this purpose, and until the recent past the birth/death cycle of nature amply fulfilled this need. But science unwittingly destroys this awe-inspiring element. The explanations of the Sun rise, eclipses and procreation no longer invoke the pantheon of the gods, but can be reduced to mathematical formulae and test-tube demonstrations.

We might well pause to reflect upon the consequences of this loss and the atrophying of our sense of wonder. As Ecclesiastes says: "In much wisdom is much grief: and he that increaseth knowledge increaseth sorrow". (1:18)

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SIR—Following the Benveniste saga, perhaps the time has come for the creation of an independent body to scrutinize laboratory work during the early stages of research.

One possibility would be the borrowing of methods employed by agencies such as the Inland Revenue, namely the tactic of the hit squad. A roving gang of suitably qualified scrutineers would descend unannounced upon unsuspecting laboratories, ruthlessly checking routines for the inclusion of relevant sampling and statistical errors. Spurious research would thereby be eliminated at an early date, benefiting researcher and reader alike, while more controversial research would be subject to the rigours of investigation normally reserved only for those working outside the scientific paradigm.

In fact, if the Inland Revenue inspectors could be persuaded to undertake such a task, a double benefit could be had in the discovery of any financial irregularities within the chosen laboratories.

The creation of such a system would

provide the suitable climate of moral fear and financial accountability under which basic science is expected to operate.

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SIR—Setting aside for a moment the issue of the scientific reliability of the Benveniste work, I wish to add my voice to those who have been dismayed by the approach to this case taken by *Nature*. The elements of farce, witch hunt and arrogance noted in the press should have been readily appreciated in advance — I would hope not intentionally — as a way of teaching anyone a lesson. This affair does not serve the cause of science, and most particularly not the cause of science's long-suffering public image.

Regrettably, it is typical of the lack of self-reflection of 'establishment' science, and reactionary response to much that is on the border of 'new science'. Galileo, Kerkule, Tesla and Einstein all survived, Reich died in prison labelled a fraud by the US Food and Drug Administration. How much of what is published as 'science' in modern journals would survive exhaustive self-delusion tests? How much is ever repeated, especially when large expenses are involved?

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World of guesses

SIR—I am surprised at your continuance of the "creationism and science" correspondence. The writers of such letters on both sides of the debate have singularly failed to realize that:

- (1) what we call knowledge (or science) is only a variably tested guess at what exists outside the reference point for certainty, which is consciousness of oneself;
- (2) the words proof, truth and objectivity do not apply in the world of guesses;
- (3) we work as 'scientists' to increase the confidence we can place in those guesses that describe and/or explain that which seems to exist beyond and within the self;
- (4) explanations that involve concepts of 'gods' or other 'supernaturals' are guesses as other guesses are; the extent to which they are useful guesses is determined by the degree to which they can be reliably applied to arrive at knowledge of the non-self world and thereby provide the guidelines for building systems of morals.

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Crystals from first principles

A new calculation of the polymorphs of silica appears to have broken new ground in deriving crystal structure from chemical composition. But X-ray crystallographers need not worry — yet.

ONE of the continuing scandals in the physical sciences is that it remains in general impossible to predict the structure of even the simplest crystalline solids from a knowledge of their chemical composition. Who, for example, would guess that graphite, not diamond, is the thermodynamically stable allotrope of carbon at ordinary temperature and pressure? Solids such as crystalline water (ice) are still thought to lie beyond mortals' ken.

Yet one would have thought that, by now, it should be possible to equip a sufficiently large computer with a sufficiently large program, type in the formula of the chemical and obtain, as output, the atomic coordinates of the atoms in a unit cell.

That time may not yet have arrived, but S. Tsuneyuki, M. Tsukada and H. Aoki from the University of Tokyo, with Y. Matsui from Okayama University, have brought it a good step nearer. Starting with the information that the polymorphic forms of pure silica consist exclusively of SiO_2 , they first calculate, from first principles, the force constants in clusters of the atoms and then, by molecular dynamics, the lattice constants of the four common polymorphs of silica (α -quartz, α -cristobalite, coesite and stishovite — the two last first recognized in the debris from meteoritic impacts).

The authors thank two computer centres in Japan for access to computing machinery, although they do not say how many Cray-equivalent hours their calculations occupied (*Phys. Rev. Lett.* **61** 871; 1988).

The calculation is not merely a taste of things to come but a pointer to the obstacles that remain, as well as being, in its own right, a considerable achievement. What the group has done is to invert the usual procedure in which force constants are calculated empirically from the properties of crystals. Their strategy is instead to calculate the force constants from first principles, for which purpose they must calculate the electronic structure of a complex of silicon and oxygen atoms.

The snag is that, even with the the price of Cray machines and their equivalents falling year by year, it remains beyond the capacity of computation to calculate the properties of a real crystal. So Tsuneyuki *et al.* start with the simplest of all relevant structures — the tetrahedral cluster SiO_4^{4-} .

But why SiO_4^{4-} , not SiO_4 ? Because Si readily surrenders electronic charge to

oxygen atoms — the authors estimate 0.7 of an electron charge for every SiO bond, or close on two electron charges for those O atoms bound to two Si atoms. So, to ensure that their calculated structures are electrically neutral, the authors place electronic charges at points 1.65 Å along the four tetrahedral axes of the cluster, neatly simulating in the process the electrostatic effects of the neighbouring oxygen ions found in structures such as quartz.

It would be wrong to say that, from this point, everything depends on what the programmers have done, but that is how the article reads. Tsuneyuki *et al.* essentially calculate the electronic structure of the cluster for various departures of the atoms from their mean positions. They use pre-calculated atomic orbitals in a Hartree-Fock self-consistent field procedure (one of the means of allowing for electron-electron interactions in many-electron atoms).

In principle, there is every reason why the energy of the system should be quite a complicated function of the atomic positions. The surprise is that the parts of the energy surface so far calculated can be accurately represented by potential functions depending only on the distances between pairs of atoms in the cluster. Not surprisingly, one of these is a Coulomb term, another corresponds to the van der Waals attractive potential proportional to r^{-6} where r is the separation.

The molecular dynamics is made to seem just routine, no doubt because of the careful development of the appropriate programs by Japanese colleagues. Pressure and temperature are determined by the size of a repeating three-dimensional unit in a periodic lattice and by total kinetic energy respectively. The known structures of the four polymorphs of silica are each dealt with separately. In each case, the object of the exercise is to tell whether the polymorphs are dynamically stable, or that they are not spontaneously converted into each other, which all four prove to be. This is taken as an explanation of why they are all apparently stable under ordinary conditions even though, thermodynamically, they are metastable.

Given previous difficulties in calculating from first principles the properties of real solids, the results are extremely good. Unit cell dimensions turn out to be within 5 per cent of their measured values, bulk properties such as density and bulk

modulus within 10 per cent. The agreement between calculated energy (enthalpy) of the polymorphs and the few measurements available is even better (1 per cent), but the authors say they could probably do even better if they allowed for the band structure of these polymorphs and the extent to which the bands are filled at different temperatures.

Several refinements remain to be explored. The chances are that three-body potentials and even more complicated variations of energy with configuration can be demonstrated by calculation and by comparison, for example, with measured specific heats. Evidently the treatment of charge transfer between Si and O, while effective, is somewhat artificial and does not allow for cooperative configurations extending over more than one SiO_4^{4-} tetrahedron.

In themselves, of course, these results are not a fulfilment of the goal of calculating from first principles the crystal structure of a compound of which nothing is known except chemical composition. But that cannot be far away.

On the face of things, the most obvious artificially introduced variables are the known crystal structures of the polymorphs of silica, used as a starting point for the molecular dynamics. It should not be beyond the wit of Tsuneyuki and his colleagues, with the help of a few computer centres, to select from a listing of all possible structures of an arbitrary material those that can be considered plausible on crystallographic and other grounds.

Perhaps the most striking feature of this laconic paper is the contrast it presents with the state of the art of calculating the properties of solids as recently as a decade ago, when it seemed that even the use of force constants derived empirically from the measured properties of crystals did not regenerate, on calculation, the authentic properties of those same crystals. But the practical importance of the new calculation could be considerable, especially with the need to learn something of the crystal structure of materials that are either very small ('microdot' zero-dimensional quantum wells, for example) or which vary in composition over distances smaller than the limits of X-ray crystallography (such as doped semiconductors). In applying calculations to these needs, a demonstration of success can rank, psychologically, with the example set by those who first climbed Everest. **John Maddox**

Solar astrophysics

Whirlpool traced by granulation

Juri Toomre

OUR Sun displays highly turbulent flows in its surface layers. Seen in white light, its surface is covered with a changing tessellated pattern of small convection cells called granules. These granules are temporary structures of hotter fluid which evolve on timescales comparable with the flow travel-time across these cells. Exceptional high-resolution observations reported by Brandt *et al.* on page 238 of this issue¹ reveal granules being swept into a strong whirlpool in what otherwise appears to be a quiet region of the Sun. Thus the granules are almost like corks carried along by an underlying swirling flow. If the Earth's atmosphere has hurricanes and tornadoes, and its oceans large eddies, and Jupiter has the Red Spot, then surely the Sun might also possess discrete vortices? Indeed, turbulence constrained by rotation and stratification is likely to exhibit coherent structures possessing strong vertical vorticity. Yet this is the first clear observational evidence of strong vortex flow in the Sun. One reason for this is the difficulty of ground-based measurement of granules because of blurring and image distortions by Earth's atmosphere.

The discovery of the whirlpool by Brandt *et al.*¹ was achieved by techniques of real-time image selection pioneered by Scharmer² which herald new possibilities for high-resolution solar observations from the ground. Tracking the movement of the granules (see figure) requires sampling only the best instants of seeing. This was made possible using charge-coupled device (CCD) array detectors and fast

real-time image processors. Further, the meticulous design of the new Swedish solar telescope on La Palma used by Brandt *et al.* leads to very low scattered light levels and remarkably high-contrast images³. The swirling flow of granules reveals that substantial helicity (the vector product of velocity and vorticity) is present in solar turbulence on granular and mesogranular scales (see below). This is an important finding, for dynamo theory advises that helicity is the crucial ingredient if magnetic dynamo action is to proceed in a turbulent plasma. Such innovative observing and analysis techniques point the way to unravelling some of the intricacies of turbulence in the convection zone of our nearest star.

Much of the roiling and boiling motion near the solar surface results from thermal convection which transports nearly all of the solar energy flux throughout a zone extending almost a third of the way into the interior. That convection also builds, shreds and organizes magnetic fields into active regions and networks of strong fields; it also redistributes the angular momentum, causing the Sun to rotate differentially. Thus there is ample reason to study solar convection by detailed observation, seeking to establish the spectrum of such turbulence and its ability to build coherent large-scale structures.

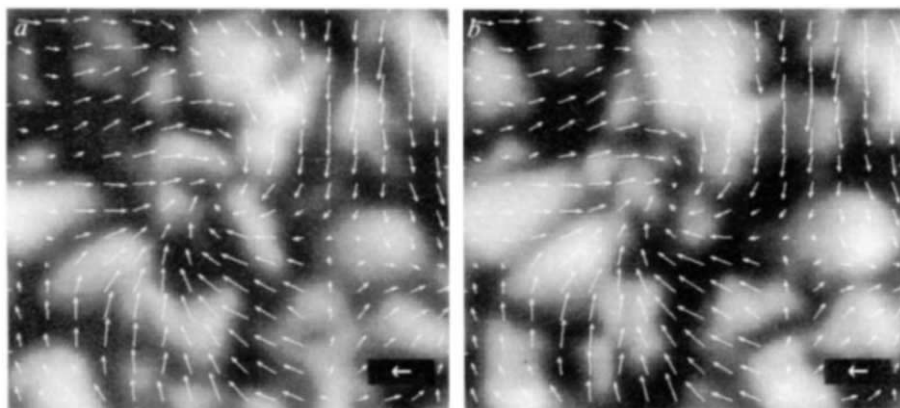
The interpretation of solar turbulence is not simple, as it contains several classes of seemingly distinct cells. The observed convection cells in the surface layers range in size from small but vigorous granula-

tion, with horizontal scales of about 1,000 km, to mesogranulation (7,000 km) and supergranulation (30,000 km). Nobody yet knows why three scales of cellular motion should be picked out in the convection in the Sun. One explanation is that they could arise from preferential driving of successively larger cells by the three distinct ionization zones of hydrogen and helium. Another possibility is that the turbulent power spectrum is more continuous than the apparent cells may suggest, with the energy cascade proceeding from supergranular to granular scales⁴. Such issues rest on nonlinear properties of compressible flows spanning many density scale heights, and these are just beginning to be understood theoretically^{4,5}.

Also puzzling is why the giant convection cells, deduced from numerical simulations^{6,7} to span much of the 200,000-km depth of the solar convection zone, have eluded detection as flows near the surface. Such north-south-oriented columnar cells are clearly evident in laboratory experiments on Spacelab 3 of convection in rotating spherical shells⁸. One possibility is that effects of compressibility and shrinking scale heights in the Sun deflect laterally the vertical flows of the largest convection cells as they approach the surface from below, producing strong horizontal flows beneath the photosphere. The giant cells could thereby be prevented from roaring into the atmosphere. Such deflection operating in a weaker manner could also explain the prominent horizontal flows, and the feeble vertical ones, observed in supergranulation.

Elucidation of how the entire convection zone works is not straightforward, as the turbulence involves a very broad range of scales and the flows are decidedly compressible. Dynamical processes deep in the zone are still uncertain, and more observations are needed. Interesting velocity fields may lurk there, whether they be shearing flows from deflected giant cells or even persistent zonal jets. Helioseismology affords one route to search for such subsurface flows, using frequency splittings of the 5-min oscillations⁹. To such ends, the GONG project¹⁰ is developing a network of ground-based Doppler imaging instruments at sites around the globe to permit continuous observation of low and intermediate-degree ($l \leq 200$) oscillation modes. The network should become operational in a few years' time.

Probing flows with high-degree oscillations will require observations from space to avoid atmospheric distortions. Starting in 1995, the Solar Oscillations Imager (SOI) on the SOHO spacecraft will yield such data, providing continuous monitoring of high-degree modes (with $l \leq 700$ over the full solar disk, and up to 4,000 locally). This joint ESA-NASA spacecraft will be placed in a halo orbit at the



Vigorous turbulent convection near the surface of the Sun has a cellular appearance at the smallest scales visible through the Earth's atmosphere. The granulation cells involve a range of horizontal sizes, but 1,000 km is typical. The granules can be carried along by underlying flows of larger scale, though such lateral advection may be difficult to discern since a given cell evolves rapidly during its typical 10-min lifetime. The figure shows a pair of solar white-light images separated by about 2 min in time (*a* is earlier), obtained from a portion of the field of view analysed by Brandt *et al.*¹. The persistent swirling flow they discovered is indicated by the arrows, with such velocities determined from cross-correlating many individual full images after destretching them to correct for atmospheric seeing distortions. Large arrow, flow speed 2 km s^{-1} ; images $5,100 \text{ km}$ ($7''$) in width. Flow into the whirlpool is suggested by the apparent displacement with time of the centres of many granules.

langrangian point L_1 , both to be in continuous sunlight and to minimize orbital Doppler velocities relative to the Sun. The two helioseismological efforts are complementary, with GONG capable of probing differential rotation with depth and latitude, and SOI capable of studying more detailed dynamics of giant cells, zonal jets and smaller-scale flows closer to the surface. Several other helioseismological observations are also either under way or planned¹¹. Inversion of the various oscillation data will be challenging, but from them should emerge a mapping of large-scale velocity and thermal structures throughout the convection zone.

Another promising route for examining flows near the surface involves high-resolution observation of granulation, tracking the movements of the cells as they evolve (see figure). Because the granules are convection cells confined to a shallow layer range near the surface, the plasma in which they reside should be swept along by the larger scales of motion that are deeper seated and more persistent. Such apparent advection of granules by flows of both mesogranular and supergranular scales is beautifully shown in the analysis¹² of white-light images obtained with the SOUP instrument on Spacelab 2. Being closely packed and fairly uniformly distributed, the granules can serve as excellent flow tracers. However, granules typically have lifetimes of only about 10 min, and thus tracking them requires frequent sampling of images and subtle pattern recognition algorithms. Further, provision must be made to filter out velocity and intensity contributions from the solar 5-min oscillations which would otherwise obscure the combined advection and evolution of granules. Analysis of the Spacelab 2 data¹² reveals the potential of using granules to track larger-scale flows, and other high-resolution solar observations from space are being planned.

Ground-based observations of solar granulation are severely impeded by blurring and image distortions resulting from turbulence in the Earth's atmosphere. Despite such difficulties, the impetus to study the smallest scales of turbulence visible on the Sun is great. In particular, the granular flows are energetic and capable of shuffling and twisting the magnetic fields in a manner that should lead to field reconnection and heating of the upper atmosphere. This notion is supported by time sequences of granulation images obtained using special telescopes when atmospheric seeing is at its best at sites such as Sacramento Peak and Pic du Midi, choosing only the sharpest images out of rapid bursts of photographs. Real-time image selection followed by digital processing to correct for distortions, as used by Brandt *et al.*¹ at the Swedish telescope, point the way for future studies of solar granulation. □

Fungi not fire damaged Aleppo Codex

THE Aleppo Codex, the earliest known manuscript of the Hebrew Pentateuch (the first five books of the Old Testament), was damaged by a fungus of the genus *Aspergillus*, rather than by fire as was previously assumed. The Codex was written on parchment in Tiberias in the tenth century. After a period in Jerusalem and Egypt, it was kept for 600 years in a synagogue in Aleppo (Halab), Syria. In 1947, the synagogue was set on fire



A page from the Codex showing typical discolouration and deterioration in the outer lower corner. Inset: fluorescence photomicrograph of fungus-like filaments in the damaged areas. Preparations mounted in the fluorescent fungal stain Cellufluor (Polysciences), KOH and glycerol. $\times 600$.

but 295 pages, about two thirds of the original manuscript, survived and were given on permanent loan by the Jewish community in Aleppo to the Israel Museum for restoration and exhibition. The fate of the missing pages is unknown^{1,2}.

The outer lower corners of all the pages are aubergine-coloured and brittle (main figure) and the parchment is weakened in these areas, which were subject to constant handling by people turning the pages. This pattern is more characteristic of damage by fungi than by fire³, and early signs of deterioration were already visible in 1910⁴. Itzhack Polacheck and colleagues* have now examined fragments from the damaged areas with phase-contrast and fluorescence microscopy, which reveals positive fluorescent septate filaments (inset) not seen in fragments from the undamaged areas. Specific fluorescent-antibody staining identifies the filaments as *Aspergillus* hyphae. □

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Oxide superconductors

Contrasts in critical current

David Caplin

How superconducting are the new high-temperature superconductors? When sufficiently small currents are passed through them, and in the absence of a magnetic field, their resistance is zero (or so low that circulating currents do not decay for days or weeks). But the superconducting transition broadens severely in a strong field (Fig. 1), and the small but finite residual resistance in the long low-temperature tail is a significant obstacle to applications of the new materials. Equivalently, the critical-current densities are disappointingly low (Fig. 2.) If they are to be useful, zero resistance is needed at 77 K (the temperature of cheaply available liquid nitrogen) for large current densities and in high magnetic fields. Conventional superconductors, which of course have to be used at liquid-helium temperatures, show a much more abrupt transition to the resistanceless state, and the contrast between the two groups of materials was the main focus of a recent meeting*.

When the superconducting Bi- and Ti-based oxides were discovered earlier this year, because their transition temperatures (up to 125 K) were significantly further above 77 K than the now 'classical' $\text{YBa}_2\text{Cu}_3\text{O}_7$ ($T_c = 92$ K), there were hopes

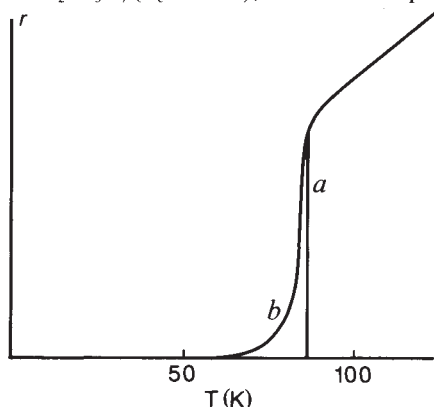


Fig. 1 Schematic resistive transition of $\text{YBa}_2\text{Cu}_3\text{O}_7$, *a*, in zero field; *b*, in a field of 10 tesla. The resistive tail in *b* arises from the motion of magnetic flux induced by the Lorentz force between current and flux; it can be eliminated only by pinning the flux more strongly.

that their critical-current performance would be significantly enhanced. Unfortunately this appears not to be the case, for reasons that are now beginning to be understood.

There are two important factors that limit the critical-current density J_c of the new superconductors: the presence of 'weak links' at grain boundaries (and perhaps within grains also); and the

weakness of flux pinning. Elegant experiments on thin films of $\text{YBa}_2\text{Cu}_3\text{O}_7$, grown epitaxially on strontium titanate (J. Mannhart, IBM Yorktown Heights) show that although the measured critical currents within a single grain can be extremely high, the inclusion of a grain boundary causes J_c to drop drastically; even misorientations of a few degrees in the *a-b* plane (which should be relatively harmless compared with misorientations with respect to the weakly conducting *c*-direction) bring about a tenfold reduction. Further, the grain-boundary J_s are about three orders of magnitude more field-sensitive than J_s in a grain; fields of only a few millitesla suffice to suppress the former.

Why do grain boundaries do so much damage? Some microstructural studies (D.M. Kroeger, Oak Ridge National Laboratory) suggest that there are deviations from stoichiometry on a 10-nm scale, but at an atomic level, the local structure at a grain boundary is certain to differ from that in the bulk. A conventional superconductor is insensitive to these local perturbations because its superconducting coherence length ξ is orders of magnitude greater than interatomic spacings. In contrast, in the new superconductors ξ is little larger than atomic dimensions, so that the grain-boundary region can interpose a layer a few nanometres thick of what is effectively a non-superconductor. A great deal can be learnt from comparing the behaviour of the new materials with that of arrays of conventional superconducting islands connected by weak links (usually a normal metal); because the relevant lengths are much larger, these arrays can be fabricated by microlithographic techniques (P. Martinoli, University of Neuchatel).

One way to circumvent the weak-link problem is to eliminate grain boundaries entirely; carefully grown epitaxial films of $\text{YBa}_2\text{Cu}_3\text{O}_7$ do appear satisfactory in this respect (A. Kapitulnik, Stanford University). Progress is being made also in achieving highly textured bulk materials, with aligned grains up to 10 mm in length, and with J_s up to 25,000 A cm^{-2} in zero field, dropping by a factor of four or so in 1 tesla (S. Jin, Bell Labs, Murray Hill).

Flux pinning is vitally important because the Lorentz force between the transport current and the flux lines causes the latter to move, so generating a voltage, unless they are pinned. Several experiments have measured the strength of pinning: the time- and field-dependence of the magnetization (Y. Yeshurun, IBM Yorktown Heights); the a.c. susceptibility

(R. M. Yandofski, IBM Yorktown Heights); the form of the low-temperature tail in the resistance (T.T.M. Palstra, Bell Labs, Murray Hill); and the mechanical damping associated with flux line motion (D.J. Bishop, Bell Labs, Murray Hill). Although the numbers that emerge show a broad range, they are all below about 0.1 eV, which is an order of magnitude smaller than in conventional superconductors. Also, it seems that pinning in BiSrCaCuO and TiSrCaCuO systems is a lot weaker than in $\text{YBa}_2\text{Cu}_3\text{O}_7$; perhaps the high density of twin boundaries in the

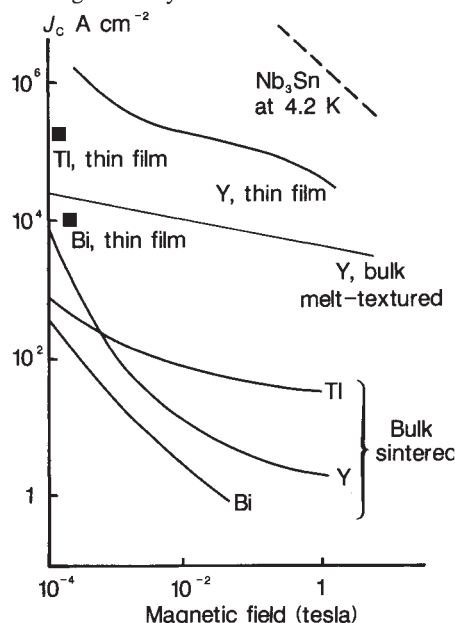


Fig. 2 Summary of recently reported critical current densities at 77 K in the new oxide superconductors. Care is needed in comparing data because of the different criteria used to define the critical current. Y, $\text{YBa}_2\text{Cu}_3\text{O}_7$; Bi, BiSrCaCuO ; Ti, TiSrCaCuO of variable compositions. The performance of the conventional superconductor Nb_3Sn at 4.2 K is shown.

latter is helpful here. General arguments suggest that the shortness of the coherence length leads inevitably to weak pinning. In any case, if the new materials are to be used at liquid-nitrogen instead of liquid-helium temperatures, the 20-fold increase in temperature greatly increases the thermal activation of flux motion.

Work reported at the meeting shows what can be learnt from two decades of experience with conventional Type II superconductors, but also highlighted the essential differences of the new materials: in the former, structural defects are beneficial — they pin flux; in the latter, they both depress superconductivity and fail to pin flux effectively. Unless ways can be found to overcome the weak-link problem and to pin flux more strongly, the prospects for high-current applications of the new superconductors look poor. □

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* Critical currents in high-temperature superconductors, Snowmass Village, Colorado, 16-19 August 1988.

Oceanography

Ocean productivity from space

Paul Falkowski

THE central ocean basins are sometimes characterized as miles and miles of miles and miles. Shipboard observers of the nutrient-poor waters of the open oceans have frequently commented on the weak horizontal gradients in chemical and biological properties, compared with the continental ocean margins. The lack of horizontal gradients has hampered the validation of empirical mathematical models that relate phytoplankton pigments, as perceived by satellite, to primary production. By a combination of skill and luck, Lohrenz *et al.*¹ have now been able to test a model relating surface chlorophyll, obtained from the now-defunct coastal zone colour scanner aboard the Nimbus-7 satellite, to shipboard measurements of primary productivity in a normally oligotrophic ocean region, the western Mediterranean Sea. The importance of the report by Lohrenz *et al.* on page 245 of this issue is not the model *per se*, which is similar to one developed by Eppley *et al.*², but that the "precision [is] adequate to resolve, for the first time, short-term fluctuations in primary productivity with a mesoscale circulation feature".

The average primary productivity in the oligotrophic ocean basins has been keenly debated within the past decade. Although primary productivity per unit area in the central ocean basins is undoubtedly lower than it is in continental shelves and in coastal upwelling regions, the total global carbon flux is greater in the former simply because of the enormity of ocean basins. The extent to which atmospheric inorganic carbon can be sequestered in the oceans by phytoplankton photosynthesis and the subsequent sinking of particulate organic carbon is important for understanding the degree to which the oceans will mitigate the build-up of CO₂ in the atmosphere.

The introduction of satellite images of oceanic chlorophyll in the late 1970s provided the potential for observing the surface distributions of phytoplankton on spatial scales of tens to thousands of kilometres. These images revolutionized thinking and enabled biologists to address the fluxes of carbon and nutrient elements on the same spatial scales as geochemists. But the extrapolation of surface estimates of chlorophyll to estimates of aerally integrated primary production has been difficult; the variations in estimates of production derived from models of satellite images are as great as the variations between shipboard observations.

In part this problem may result from the fact that the range of production values normally measured by one investigator on

a cruise is too low to validate an empirical model. To obtain a sufficiently useful range of production values to examine the robustness of a model, data sets from various investigators, often from different environments, are mixed. The result of such data mixing is usually such a large variance in the modelled production as to render the models almost useless in rigorous calculations of carbon flux.

Lohrenz *et al.* observed that on 8 May 1986 a phytoplankton bloom had developed off the coast of western Algeria which had disappeared by 11 May. The bloom was associated with a sporadic physical mixing event: the differences between surface chlorophyll concentrations during and after the bloom spanned an order of magnitude. Observations of such a large difference in surface chlorophyll in such a brief period are rare, particularly in oligotrophic regions of the oceans. The large range of surface chlorophyll concentrations allowed Lohrenz *et al.* to use regression analysis first to relate the satellite image of surface chlorophyll, C_s , to measurements of surface chlorophyll, C_s , and second to relate C_s to aerally integrated primary production, π .

The importance of short-term transient events of the type described by Lohrenz *et*

al. to overall production has increasingly been realized^{3,4}. Whether such events significantly displace estimates of production from the mean remains to be seen. There is no satellite in orbit capable of measuring surface chlorophyll in the oceans. The coastal zone colour scanner, launched in 1978, failed to return data by the end of 1986. The next satellite planned for ocean-colour data collection is the sea wide-field sensor to be launched aboard Landsat-6 by NASA and Eosat Corp. in 1991.

The development of satellite sensors is occurring in parallel with the development of new types of instruments which can be moored for extended periods in the oceans. Instruments for measuring phytoplankton pigments and even primary productivity based on variable fluorescence can be moored in different locations for use as a base for the development of satellite algorithms⁵. It is to be hoped that the combination of satellite images and moored instrumentation will eliminate the 'luck factor' in observations of transient mixing events. If so, by the end of the century we should have a clear consensus about the magnitude and importance of oceanic production in the global carbon cycle. □

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Solar System

Interior and origin of Pluto

David W. Hughes

OUR knowledge of Pluto is increasing painfully slowly. At the time of discovery (1930) astronomers were shocked at its faintness and its low mass. It was not the planet 'X' for which they were searching in the hope of explaining Neptune's perturbed orbit. Pluto's orbit is inclined at 17.2° to the plane of the Solar System and it has an eccentricity of 0.250, a value that brings it within the more circular orbit of Neptune. Naturally, astronomers have speculated that Pluto has an unusual origin, perhaps as the escaped satellite of an inner planet. Elsewhere in this issue (*Nature* **334**, 240–243; 1988), W. B. McKinnon and S. Mueller use the measured mean density of Pluto and its moon Charon to constrain the possible models of Pluto's interior and hence its origin.

Since 1985, and until 1990, Pluto and Charon have been undergoing a series of mutual eclipses, a phenomenon that occurs twice every 248 years. The timing of these eclipses gives accurate values for the

radii of Pluto and Charon, $1,122.7 \pm 3.5$ and 599.7 ± 5.8 km, respectively. Charon is tidally locked to Pluto and has the same face always pointing towards it. It orbits every 6.38718 days, so that the system has a total mass of 1.36×10^{25} g, a mere 18.5 per cent of the mass of our Moon. Together, Charon and Pluto have a mean density of 1.991 ± 0.018 g cm⁻³.

If Charon has a density of $1-3$ g cm⁻³, a range that spans all the known values for icy satellites, then the density of Pluto lies between 1.84 and 2.14 g cm⁻³. So Pluto must be extremely rocky. In fact, with these densities, rock makes up 68–80 per cent of its total mass. McKinnon and Muller use models of Ganymede (radius 2,638 km) and Callisto (radius 2,410 km) as their guides, but with one important proviso: Pluto is so small that its gravitational pressure has not significantly increased the rock density in its interior over its atmospheric-pressure value.

They also consider two types of rock.

One is similar to carbonaceous chondrites, the most primitive type of meteorites. The other has a solar abundance of the rocky elements, with H_2O added. The rock in the core of Pluto must be hydrated, this state being produced by the accretion of silicates and warm water-ice in the early stages of the growth of the planet. And as Pluto is so small, the heating by subsequent radioactive decay will not have increased the temperature up to the 800–1,200 K required for dehydration.

Two possibilities arise. Either Pluto is undifferentiated, or it has a large silicate core surrounded by a mantle of ice 200–300-km thick. Unfortunately, constrained by the facts given above, both models lead to very similar rock to rock-plus- H_2O mass ratios. McKinnon and Mueller, however, favour differentiation. The presence of methane on the surface of Pluto supports this view, as do calculations of the energy released during the accretion process. Heating by the radioactive decay of uranium, thorium and potassium also helps. It seems that Pluto contains more silicates than all other icy satellites in the Solar System except Europa. This high rock-to-ice ratio provides an important clue as to the origin of Pluto. McKinnon and Mueller consider four possibilities.

In one, Pluto was formed in the inner Solar System (Mercury–Mars) and was then gravitationally perturbed by the other planets into its present orbit. McKinnon and Mueller dismiss this hypothesis because it fails to account for the presence of so much ice (this would have evaporated so close to the Sun). Further, the perturbation would probably disrupt the delicate Pluto–Charon system.

Second, Pluto may have been formed in the Jupiter–Saturn region. But there, large satellites have a rock/water-ice ratio near the cosmic value of 40/60, and the gravitational potential energy of the accreting Pluto is insufficient to remove great amounts of H_2O .

Third, Charon might have been formed by a collision between Pluto and an outer-Solar-System asteroid, a rather improbable event which could have resulted in different end points. As objects in the outer Solar System are, in general, moving slowly, such a collision would be of low energy. The resulting fragments would probably have insufficient energy to escape from the gravitational potential-well of the primary body, but they could have reassembled into a binary. If the protoplanet had differentiated before the collision, the binary components could have different densities. Considerable amounts of volatiles could have been lost in the process. If the collision took place near Neptune, this scheme would also explain Pluto's peculiar orbital relationship with that planet.

The fourth possibility is that Pluto is one of the largest members of a class of outer-

Solar-System asteroids. In the cold outer regions of the pre-planetary nebula, carbon could have locked up much of the oxygen in the form of carbon monoxide and this would automatically increase the rock-to-ice ratio in any planetesimals that formed. The upper limit obtained is 73 per cent rock to 27 per cent ice, a value close to that found by McKinnon and Mueller for Pluto. Pluto would have to be formed way beyond Saturn and Uranus because in the Saturn–Uranus region the ice content of satellites is much larger.

Unfortunately, the authors cannot decide between the last two possibilities and think that both might be needed,

resulting in an outer planetesimal which has also suffered a collision. But the 7-to-1 mass ratio between Pluto and Charon is very high for a binary and is much more typical of the breaking up of a rotating molten body that becomes unstable as it condenses and spins faster through conservation of momentum. On the other hand, a collision is rather like an explosion: the fragments can take away considerable amounts of energy, certainly enough to explain Pluto's odd orbit. □

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Nuclear energy

Pellets feel the pressure

R. S. Pease

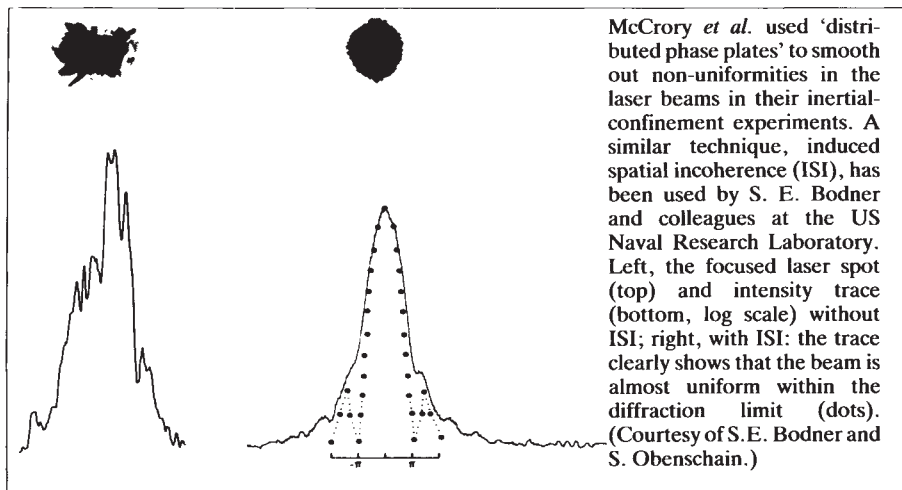
NUCLEAR fusion, the merging of two nuclei to form a new nucleus, releasing energy, occurs in materials at high density and temperature. One scheme used by nuclear engineers hoping to obtain net energy release by fusion is to compress a pellet of fuel using heat generated by a laser pulse — inertial confinement. R. L. McCrory *et al.* report elsewhere in this issue (*Nature* 335, 225–229; 1988) the highest density of nuclear fuel yet achieved by the direct use of laser radiation, 20–40 g cm⁻³.

Since the military demonstration in 1952 of the large-scale explosive release of nuclear-fusion energy, research has been undertaken to see if the energy could be released in a controllable form by generating small-scale explosions. To keep the pulsed energy release to a containable amount — say up to about 1,000 MJ — it is necessary to compress the fuel of the hydrogen isotopes deuterium (D) and tritium (T) to very high densities, typically to 1,000 times their liquid density. In a scheme proposed by J. Nuckolls *et al.* (*Nature* 239, 139–142; 1972) radiation

from pulsed lasers is focused on the surface of small (about 100 μm across) hollow glass spheres containing DT fuel, either gaseous or in a layer frozen onto the inner wall. The laser radiation heats the surface to millions of degrees, thus creating pressures in the 1–100-megabar range which implode the sphere.

The first experimental results (Brueckner, K. *et al.* *Nucl. Fusion* 15, 471; 1975) indicated the general feasibility of the approach. The final density depends on achieving a high degree of spherical symmetry in the implosion: a sphere of liquid DT compressed to a tenth of its initial radius, for example, would achieve the required densities, but only if the radial collapse is uniform, which in turn requires uniform pressure.

The densities of 100–200 times liquid density reported by McCrory *et al.* in this issue have been achieved by giving special attention to securing uniformity of the radiation power over the surface of the sphere. The OMEGA laser facility at Rochester University that they use consists of 24 separate beams of laser radi-



McCrory *et al.* used 'distributed phase plates' to smooth out non-uniformities in the laser beams in their inertial-confinement experiments. A similar technique, induced spatial incoherence (ISI), has been used by S. E. Bodner and colleagues at the US Naval Research Laboratory. Left, the focused laser spot (top) and intensity trace (bottom, log scale) without ISI; right, with ISI: the trace clearly shows that the beam is almost uniform within the diffraction limit (dots). (Courtesy of S. E. Bodner and S. Obenschain.)

ation, each effectively subdivided into about 10,000 beamlets by inserting in each beam distributed phase plates made from elements of slightly varying optical thickness. This induces spatial incoherence in the phases and spreads the beamlets, reducing the non-uniformities of the radiated power falling on the surface to less than 12 per cent. A similar improvement to the radiation uniformity in the focal point of a laser (see figure) was recently described by S. E. Bodner (US Naval Research Laboratory, unpublished).

The experiments of McCrory *et al.* are distinguished also by the use of a new direct method of measuring the compressed fuel density, namely by counting the nuclei knocked out of the collapsed sphere by the 14.1-MeV neutrons generated by nuclear-fusion reactions. The number of knock-on deuterons and tritons, identified by their tracks in small polycarbonate foils near the target, is directly proportional to the total neutron output and to the mean product of number density and radius of the compressed DT during neutron emission. The lower limit to the density is obtained by assuming that the DT fuel is conserved during compression.

The compressions observed are about the same as those reported from experiments using the Nova laser facility at Lawrence Livermore Laboratory (Storm, E. *Nucl. Fusion Suppl.* 3, 15–24; 1987). But these results require more energy in the laser pulse, about 20 kJ compared with 1.5 kJ from Rochester, and use the 'indirect' method in which the laser radiation is converted into black-body radiation at the target to secure uniformity of illumination. Other direct-radiation experiments at Livermore yielded comparatively small compression of a few times liquid density, although they did yield the highest fusion output, about 30 J.

The Rochester experiments thus reopen the prospects of the original simple irradiation scheme proposed in 1972. Nonetheless, there are still hurdles to be overcome to realize the scheme in its ideal simplicity. The compression achieved by McCrory *et al.* is 2–5 times less than that calculated in the ideal model, and the nuclear-fusion energy yield, below about 3×10^{-4} J, is one-thousandth of that expected, indicating a shortfall of temperature as well. The authors tentatively attribute this to the remaining non-uniformity of the target in the illumination. If this can be reduced, it will be possible to find out whether fluid-dynamical Raleigh–Taylor instabilities, which commonly occur when a dense fluid is accelerated by the pressure of a less dense one, impose a further limit on the spherical implosion of small DT-containing glass spheres. □

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Cell biology

A mitotic inducer matures

Andrew W. Murray

Two recent papers^{1,2} suggest that the long and often difficult courtship between cell-cycle geneticists and biochemists has come to a happy ending. Two sets of collaborators each concludes that one of the subunits of maturation promoting factor (MPF) of the amphibian *Xenopus*, a biochemically characterized inducer of mitosis, is the product of the *Xenopus* equivalent of *cdc2*, the mitosis-inducing gene of the fission yeast *Schizosaccharomyces pombe*. This exciting conclusion is strengthened by the fact that the two groups reached it using different methods.

MPF is a mitosis-inducing activity present in cells that are in mitosis or meiosis. It was first recognized, and has been most extensively studied, in meiotic *Xenopus* oocytes. After many years of intractability, MPF has now begun to reveal its secrets: it is a phosphoprotein³; in interphase of both oocytes and mitotic cells it exists as an inactive form called preMPF; and there is an inhibitor of preMPF activation present in oocytes and embryos⁴. Most important, Lohka *et al.* have recently been able to purify MPF to near homogeneity⁵. The most purified fraction contains two main polypeptides of relative molecular mass 45,000 (45K) and 34K, and has protein kinase activity for several substrates, including the 45K protein. The putative small subunit is the same size as the product of the *cdc2* gene ($p34^{cdc2}$), which also has protein kinase activity. In collaboration with Nurse and colleagues, Maller's group now shows¹ that the 34K protein in the most purified fraction of MPF is recognized by antibodies raised against a strongly conserved peptide of $p34^{cdc2}$, and they conclude that this cross-reacting 34K protein (p34) is the *Xenopus* homologue of $p34^{cdc2}$.

Much of the *Xenopus* p34 is discarded in the first step of MPF purification, and this discarded material has a slightly faster mobility on gels than the material found in the active MPF (ref. 1). This raises the possibility that the p34 in MPF is phosphorylated and that this phosphorylation is required for binding of the 45K protein. More direct evidence for such a scheme comes from studies on mammalian cells, where phosphorylation of p34 (the mammalian homologue of $p34^{cdc2}$) has been demonstrated to increase during the cell cycle; only the most phosphorylated form is capable of forming a complex with an unknown 62K protein. The protein kinase activity of the p34/p62 complex increases as cells enter mitosis⁷, making it tempting to speculate that it represents the mammalian form of MPF. Free p34

also has protein kinase activity, both in mammalian cell extracts and in starfish embryo extracts, where Nurse and collaborators report on page 251 of this issue⁸ its purification as a kinase activity identified by its ability to phosphorylate histone H1. The H1 kinase activity of p34 from starfish increases as oocytes enter meiosis and declines as they leave it without any fluctuation in the amount of p34, demonstrating that the activity of $p34^{cdc2}$ homologues can be controlled post-translationally⁹. Russell and Nurse have identified *S. pombe* genes that appear to act either as post-translational activators (*cdc25*) or as inhibitors (*wee1*) of the mitosis-inducing activity of *cdc2* (ref. 9).

The alternative approach to characterizing MPF, adopted by Dunphy *et al.*², depends on the detailed knowledge of *cdc2* genetics. As I discussed in a previous News and Views article¹⁰, *cdc2* was originally identified as a gene whose activity is required for *S. pombe* cells to enter mitosis. Mutants in a second gene, *suc1*, can suppress certain *cdc2* mutants¹¹ and the 13K product of the *suc1* gene, p13, forms a complex with $p34^{cdc2}$ (ref. 12). Inspired by this association, Dunphy *et al.* show that added yeast p13 prevents MPF from inducing mitosis in interphase extracts of *Xenopus* eggs. Furthermore, when a crude preparation of MPF is passed over a p13 column, MPF activity is depleted and all the *Xenopus* p34 is bound to the column².

What role, if any, does p13 have in the *Xenopus* embryo cell cycle? One possibility is that it is not present in early embryos because it is involved in the system of checks and balances that are present in somatic cell cycles and absent in embryonic ones. A second possibility is that p34 is a promiscuous protein that can form complexes with various different subunits affecting its kinase activity and substrate specificity. Adding an excess of any one subunit would then cause the depletion of the complexes of p34 with other subunits and thus prevent phosphorylation of their substrates and the consequent entry into mitosis. Finally, p13 could be a physiological antagonist of MPF that inactivates MPF directly or indirectly. If p13 is involved in the inactivation of MPF in embryos, then other components must exist that act in some way to overcome the inhibitory effect of p13 on MPF activity.

One candidate for such a component would be the cyclins, a small group of related proteins that accumulate in interphase and are destroyed at the conclusion of mitosis in early embryonic cell cycles. The

kinetic behaviour of the cyclins and the ability of the messenger RNA encoding them to induce meiosis in frog oocytes has made them popular candidates for inducers of mitosis^{13,14}. A report in the current issue of *Cell*¹⁵ bestows genetic credentials on cyclin by showing that its *S. pombe* homologue is the product of the *cdc13* gene. The *cdc13* was initially identified as a gene whose activity was required for entry into mitosis. It has now been shown¹⁶ that *cdc13* mutants can allow certain *cdc2* mutants to enter mitosis, suggesting that the products of the two genes interact in the control of entry into mitosis¹.

When sequenced, *cdc13* was reported to have no strong homology to any known sequence¹⁷. But this apparent anonymity turns out to have reflected the absence of cyclin sequences from the protein sequence data banks: cursory inspection in fact shows obvious strong homology between the protein sequences of *cdc13* and clam or sea urchin cyclins¹⁵. The relationship between *cdc2* and *cdc13* suggests that MPF and cyclin will share a similar relationship. The simplest possibilities are that cyclin is a subunit of MPF or that it is

involved in the activation of preMPF. It seems likely that the power of yeast genetics, increasing ability to manipulate cell-cycle reactions *in vitro* and strong conservation of cell-cycle regulators, will enable geneticists, biochemists and cell physiologists to cooperate to make rapid progress in constructing a molecular picture of how the cell cycle works. □

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T-cell development

Accessories or coreceptors?

Charles A. Janeway, Jr

THE B lymphocyte, and in particular the secreted form of its receptor, the antibody molecule, have become familiar because of the enormous use of antibodies as molecular probes. By contrast, the T lymphocyte and the T-cell receptor (TCR) have remained relatively obscure except to immunologists, despite the importance of T cells in most immune responses¹.

The mystery surrounding antigen recognition by T cells is compounded of several factors. First, the T cell does not secrete its receptor, so soluble receptor proteins cannot be used for binding assays; rather, T-cell receptor specificity must be deduced from biological assays of some complexity. Second, the T-cell receptor does not recognize free antigen. Instead, T cells recognize fragments of protein antigens² bound to molecules of the major histocompatibility complex (MHC)³ on the surfaces of other cells. Third, normal T cells recognize antigen-MHC ligands only when the MHC molecule is of the same genotype as the T cell.

This self-MHC bias of the TCR is not encoded in the TCR genes, but rather is acquired by contact with cells bearing these MHC proteins during T-cell maturation in the thymus, the central lymphoid organ in which T cells first express their receptors and mature to immune competence. To paraphrase Marx, it is not the repertoire of inherited receptors that

determines cell interactions, but on the contrary, cellular interactions that determine receptor repertoire*.

It is assumed that T-cell receptor genes are rearranged and expressed in a random manner, and that two selective processes occurring during intrathymic development act on this random repertoire of TCRs: positive selection for cells having TCRs that will recognize antigen fragments only if presented by self-MHC; and negative selection against cells having TCRs that would confer autoreactivity on the mature T cell, a complex and apparently paradoxical process that has been a source of some confusion even to those in the field.

Much of the data on T-cell development has derived from highly complex experimental protocols, raising the question of whether these two putative selective events occur during normal T-cell development. Now, data presented in this and recent issues of *Nature*⁴⁻⁸ using new approaches to this problem strongly support the existence of both types of selection during intrathymic T-cell development, and help to clarify this area significantly.

CD4, CD8 and positive selection

Most immature thymocytes express both CD4 and CD8, whereas mature thymo-

*"It is not the consciousness of men that determines their existence, but on the contrary, their social existence determines their consciousness." (Karl Marx, *Critique of Political Economy*, 1859.)

cytes express only one or the other. On mature T cells, the class of MHC molecule recognized by the TCR is strictly associated with expression of CD4 or CD8. T cells expressing CD4 recognize antigen presented by class II MHC molecules, whose tissue distribution is limited to certain cells of the immune system, such as B cells and macrophages. T cells expressing CD8 recognize antigens presented by class I MHC molecules, which are found on most somatic cells. Although CD8 and CD4 are believed to increase the affinity of the T-cell receptor for class I and class II MHC molecules, respectively, recent data suggest that these molecules play a more direct role in MHC recognition, actually forming part of the receptor for antigen-self-MHC (see below).

Expression of CD4 or CD8 is more weakly associated with functional capabilities, CD4 T cells tending to be specialized for secretion of lymphokines that help activate other cells, notably B cells and macrophages, whereas CD8 T cells tend to be cytotoxic. How does the specific association of CD4 and CD8 expression with the MHC class specificity of the TCR arise during T-cell development in the thymus?

A first and critical question is whether thymocytes bearing receptors capable of recognizing foreign antigens presented only by self-MHC molecules are actually positively selected for maturation and export to the periphery during development in the absence of that foreign antigen. von Boehmer and collaborators^{4,5} and Sha *et al.*⁶ address this issue by preparing mice transgenic for rearranged genes encoding TCRs derived from cloned T-cell lines that express CD8 and recognize class I MHC ligands. These TCRs are expressed in a high percentage of thymocytes and T cells in the transgenic mice, since expression of rearranged receptor genes suppresses endogenous TCR gene rearrangement⁹. The most striking result in these experiments is the finding that among mature T cells only those expressing CD8 express the transgenic TCR. More impressively, in the studies of Hung Sia Teh *et al.* reported in this issue⁵, this result depends on the presence of the self-MHC molecule for which the original receptor was specific; in mice transgenic for the same receptor but expressing MHC genes that the receptor does not recognize, maturation of T cells expressing the transgenic TCR does not occur.

The authors interpret their results to mean that T cells are indeed positively selected by recognition of self-MHC in the thymus, and that only cells expressing CD8, which is required for effective recognition of self-class I MHC by the original T-cell lines from which the receptor genes were derived¹⁰, can be so selected. A second important question is also addressed by Hung Sia Teh *et al.*⁵: because they find the transgenic mice have

many CD4⁺ CD8⁺ double-positive thymocytes expressing the transgenic TCR, they infer that T cells expressing CD8 derive from double-positive cells.

Double-positive precursor cells

Although it has been widely believed that mature CD4⁺ or CD8⁺ T cells derive from a double-positive precursor, definitive evidence has been lacking and some doubt has been cast on this belief by experiments in which transfer of highly purified double-positive cells to irradiated thymus failed to reconstitute the T-cell pool. On the other hand, Smith¹¹ has obtained evidence in its favour by treating mice with anti-CD8 and inhibiting development both of CD4⁺ and of CD8⁺ T cells. A double-positive precursor to mature T cells is also suggested by recent experiments demonstrating clonal deletion as a mechanism of negative selection of potentially autoreactive T cells¹²⁻¹⁴. In these experiments, cells bearing TCRs that confer autoreactivity to class II MHC ligands are deleted not only from the class II MHC-specific CD4⁺ subset but also from the CD8⁺ class I MHC-specific subset, which is not reactive to the self-class II MHC ligand, and therefore need not be negatively selected.

The loss of T cells bearing class II MHC autoreactive TCRs from the CD8⁺ pool could be explained if negative selection were operating on double-positive precursor cells. As a further test of this question, Fowlkes *et al.*⁷ treated mice with a dose of anti-CD4 sufficient to bind all CD4

molecules on developing thymocytes. This treatment prevents the appearance of mature CD4⁺ T cells of all specificities, probably by inhibiting positive selection; but more important, it prevents the deletion of CD8⁺ T cells bearing the class II MHC-autoreactive receptor.

A very similar result has been obtained by MacDonald *et al.*, who examined a different TCR⁸. Both groups interpret their results to mean that negative selection of the autoreactive TCR requires recognition of class II MHC by both the TCR and CD4, and that it occurs at the double-positive stage of T-cell development; thus when recognition by CD4 is prevented by anti-CD4, negative selection fails and cells bearing the autoreactive receptor can mature into CD8⁺ T cells. These results are supported by the finding⁴ that double-positive thymocytes bearing the transgenic TCR developing in mice that express both the foreign antigen and the MHC for which the receptor is specific are profoundly depleted. An alternative hypothesis based on positive selection is presented below.

Timing of selective events

Data from the studies discussed above have thus been interpreted to mean that negative selection at least occurs in double-positive cells, which subsequently give rise to the singly CD4⁺ or CD8⁺ cells. It is crucial to our understanding of T-cell development to know which selective process occurs first, and in what cell popu-

lation. It is widely assumed that positive selection precedes negative selection, primarily because positive selection seems to involve an interaction between the TCR and MHC molecules expressed on thymic cortical epithelial cells¹⁵, the thymic cortex being the site in which immature double-positive thymocytes are found. Similarly, negative selection requires the presence of cells derived from bone marrow¹⁶, which are believed to be found primarily in the thymic medulla, a site occupied by more mature thymocytes.

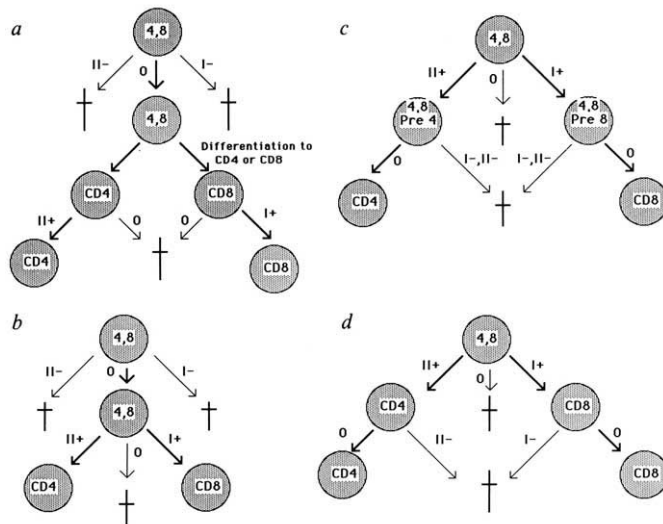
Can one explain the new results on the basis of positive selection occurring in double-positive cells, and preceding negative selection? The answer seems to be yes, as shown in the figure, which presents several possible explanations for the selective events occurring in the studies under consideration. In *a*, negative selection acts on double-positive cells, which then randomly assort into CD4⁺ and CD8⁺, after which positive selection occurs. Only cells with TCRs that are not autoreactive in the presence of either CD4 or CD8 would mature in this scheme, and then only TCRs that allowed antigen recognition in the context of self-class II MHC would be positively selected in CD4⁺ T cells. In *b* and *c*, both negative and positive selection act on double-positive cells, in either sequence. In models *b-d*, CD4 and CD8 are assumed to be critically involved in positive selection. When the TCR allows foreign antigen recognition with self-class I MHC, CD8 participates in recognition and signals the cell to repress expression of CD4, and the reverse occurs with class II MHC. In *d*, positive selection occurs in double-positive cells and selectively drives cells to the single positive state, whereupon negative selection occurs.

On the face of it, the experiments on negative selection discussed above^{7,8} are difficult to explain in this last scheme, but the difficulty can be overcome if positive selection of autoreactive TCRs is so powerful that all cells expressing such receptors for self-class II MHC are driven to CD4⁺ and are then clonally deleted; anti-CD4 would prevent this positive selective process. A similar interpretation can be made of the transgenic experiments⁴ on negative selection. At the moment, it is not possible to choose between these alternatives.

Accessories or coreceptors?

Whatever model of T-cell development is correct, CD4 and CD8 are obviously critically important in both positive and negative selection. These molecules are usually referred to as accessory molecules, a term that tends to imply a subsidiary role in T-cell function. The tight association of CD4 expression with class II MHC recognition is usually explained by saying that CD4 binds to all class II MHC molecules¹⁷,

Four schemes of T-cell differentiation and selection in the thymus. Expression of CD4 and CD8 on each cell is shown. In all schemes, recognition of class I MHC ligands requires CD8 expression, whereas recognition of class II MHC ligands requires CD4 expression. Light arrows leading to a cross indicate pathways leading to cell death. Heavy arrows show pathways leading to further development. Recognition events: 0, no recognition occurring; I, class I MHC ligand recognized; II, class II MHC ligand recognized. Selective events occurring as a result of MHC recognition denoted as + if cells are positively selected, and - if cells are negatively selected. *a, b*, Negative selection before positive selection. In *a*, differentiation of CD4⁺ CD8⁺ double-positive cells into single positive cells is assumed to occur independently of MHC recognition, while in *b*, commitment to CD4 or CD8 expression occurs during positive selection of double-positive cells. *c, d*, Positive selection precedes negative selection. In *c*, cells are committed to CD4 or CD8 expression during positive selection, but undergo negative selection while still expressing both CD4 and CD8, and can thus be deleted by either class I or class II MHC. In *d*, negative selection occurs on single positive cells, and thus CD4 cells can be negatively selected only for class II MHC recognition. Note that positive and negative selection events must differ both in the consequences of the recognition event (different signal delivered to the T cell, perhaps by different cell type presenting the ligand) and in the specificity of recognition (different ligand recognized, different affinity of receptor); otherwise, all cells would be both positively and negatively selected, and no mature T cells would appear.



increasing either the adhesion of the T cell to its stimulating cell or the affinity of the receptor for its ligand. Recent evidence strongly suggests that CD4 and CD8 are actually physical components of the receptor and, as such, contribute directly to signal transduction during T-cell activation¹⁸⁻²¹. Signalling via TCR crosslinking is potentiated by about 100-fold when CD4 or CD8 are physically associated with the TCR^{18,22}. This, in turn, means that when the TCR binds a class II MHC ligand, CD4 binding to the same class II MHC molecule lowers the threshold ligand concentration required for T-cell activation by 100-fold. This effect is of such a magnitude as to make it easy to understand why CD4⁺ T cells recognize class II MHC ligands and CD8⁺ T cells recognize class I MHC ligands. As CD4 and CD8 both physically associate with the TCR and contribute so strongly to signal transduction, it is in my view more appropriate to regard these molecules as coreceptors than as accessory molecules.

The studies discussed here extend the evidence for this coreceptor function from mature T-cell antigen recognition to intrathymic T-cell development. CD4 is essential for clonal deletion by class II MHC ligands in one set of experiments^{7,8}, and coreceptor expression is dictated by TCR specificity for thymic MHC molecules in the other⁴⁻⁶.

These studies also show that it is the specificity of the TCR that dictates coreceptor expression, rather than the coreceptor dictating TCR specificity for MHC class. It makes sense for the specificity of the TCR to govern coreceptor expression, as the TCR is the variable antigen-recognition element that must control the behaviour of the cell on which it is expressed. How the TCR dictates coreceptor expression depends on which model of T-cell ontogeny turns out to be correct. I prefer models in which the two coreceptors transduce distinctive signals during positive selection in double-positive cells. These signals would share the property of inducing T-cell maturation, but would differ in that CD4 signals would programme the cell to repress CD8 expression, and vice versa. These distinctive signal-transduction systems might also account for the functional distinctions between CD4⁺ and CD8⁺ T cells.

Questions for the future

Many issues regarding T-cell development remain to be resolved. Among these are the sequence in which positive and negative selection occur, and at which stage in T-cell maturation. To validate the results obtained using transgenic mice, further experiments, especially those using TCR genes derived from class II MHC-restricted T-cell receptors, will be needed. The postulated differences in signalling for the CD4 and CD8 coreceptors must be tested

experimentally. The location in which selective events occur is also important. Using monoclonal anti-receptor antibodies, this problem should be approachable by morphometric analysis of thymic sections, especially from transgenic mice.

Finally, all studies of thymic selection raise a fundamental question: how is it possible to select for maturation T cells that have the potential to recognize foreign antigen only if it is presented by self-MHC molecules, when the foreign antigen is not present? Furthermore, these same cells must be unable to respond to self-antigens presented by self-MHC. Note that about 99 per cent of T cells die during intrathymic development, probably because only rare cells meet these stringent criteria.

Two explanations for this aspect of T-cell development have been proposed^{23,24}. One is that positive selection is more permissive than negative selection, allowing TCRs with low affinity for self-MHC to be positively selected but not clonally deleted. Alternatively, the MHC molecules on thymic epithelium may present a set of self peptides unique to this site and specialized for driving positive selection. This model would be strongly supported if it could be demonstrated directly that self-MHC in the thymic cortex presents peptides that differ qualitatively from those presented by self-MHC in the thymic medulla and peripheral tissues.

T-cell development, and the selection of the TCR repertoire in the thymus, must be regarded as very complex processes. Nevertheless, new techniques and methods of analysis are gradually illuminating this critical and fascinating area, and it is hoped in the near future even non-immunologists will find the subject freed of its pervading mysteries. □

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Daedalus

Jogging on the spot

JOGGING, says Daedalus, is a most exhausting and unpleasant activity. It does not use up enough energy to burn off any significant amount of fat, and must benefit the body by some indirect effect. One such effect is the 'oxygen-debt' induced by strenuous exercise. The hard-working muscles outrun the lungs, and the glucose in the bloodstream is metabolized faster than oxygen can be breathed in to burn it completely. Instead, it is oxidized incompletely, to lactic and pyruvic acids. These are burnt off later, as post-exertion panting repays the oxygen-debt.

Daedalus suspects that the benefits of jogging are entirely due to regular oxygen-debt. So he is seeking to impose it on the body without the pain and strain of heavy exercise. Partial suffocation might do the trick, but is probably even more unpleasant than jogging. A neater chemical alternative would be to drink a flavoured syrup of ethyl lactate and pyruvate. These esters are hydrolysed on digestion to lactic and pyruvic acids, and of course (as a useful bonus) alcohol. Imagine the appeal of DREADCO's lactate liqueur which makes you fit as it gets you drunk! As it doesn't even contain alcohol as such, it should not attract excise duty, either.

But biochemistry is seldom that simple, and Daedalus may have to imitate the effects of exercise more indirectly. DREADCO's biochemists are immobilizing the enzymes of muscle glycolysis on a suitable porous substrate, so that blood pumped through it will be transformed just as in an exercising muscle. Packed into a small tubular reactor, with cooling-fins to dissipate the heat generated, it would form an 'artificial muscle' that could be plumbed directly into the circulation by standard transfusion techniques. Not everybody enjoys sticking needles in their arms; but once that has been done, the user of DREADCO's artificial exercise machine need only switch on the pump, and lie back in a state of induced exhaustion while the obliging machine exercises for him. A simple flow-rate control will enable him to vary his virtual exertion from a gentle trot right up to a sustained Olympic sprint far beyond his real capacity.

At last the grim agony of physical endeavour can be abandoned; the obligatory fashionable garments and stylish shoes can be thrown away, the risks of overexertion forgotten, and the life of the mind cultivated once more. For the ultimate combination of laziness and decadence, artificial exercise might best be taken while watching pornographic videotapes. The heavy breathing induced by oxygen-debt would fit neatly into the mood of the occasion.

David Jones

Accounting for cystic fibrosis

SIR—Hansson¹ speculates that if the defect in the control of apical membrane Cl^- channels in cystic fibrosis (CF) extends to the intestine, a resistance to bacterial toxin-mediated diarrhoea may confer a selective advantage to carriers heterozygous for the CF gene.

There is, indeed, direct evidence, both from our group^{2,3} and from Powell's group⁴, that in CF there is a failure of the Cl^- secretory mechanism of the small intestine. Intestinal tissue from children with CF did not exhibit an increased short-circuit current (SCC), a reflection of net

meconium ileus (STa) and two adult volunteers (CT). The electrical activity of the biopsy samples was measured using a modified Ussing chamber technique^{2,3}. As shown in the figure, five control tissues responded to *E. coli* STa with a rise in SCC of $12.4 \pm 3.2 \mu\text{A cm}^{-2}$, but no such effect was observed in three CF tissues, whose SCC fell by $2.6 \pm 1.5 \mu\text{A cm}^{-2}$ ($P < 0.05$). A similar pattern was obtained with CT: 90 min after addition of the toxin, the SCC of four control tissues had increased by $33.8 \pm 9.6 \mu\text{A cm}^{-2}$, while that of four CF tissues had fallen by $10.3 \pm 3.2 \mu\text{A cm}^{-2}$ ($P < 0.01$). Glucose induced a rise in SCC in all tissues, indicating their viability.

These findings confirm that the intestine in CF homozygotes fails to exhibit a secretory response on exposure to bacterial toxins that would normally induce a

secretory diarrhoea. This leads to the speculation that heterozygotes may have a blunted response to such toxins which would confer upon them an evolutionary advantage in the form of resistance to toxin-mediated diarrhoea. This could account for the high frequency of the CF allele. The intestinal secretory responses of heterozygotes is currently under investigation in our laboratory.

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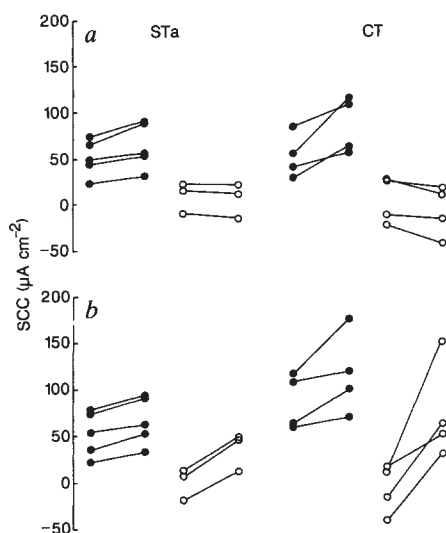
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SCC response of control (●) and CF (○) intestinal biopsy samples to a STa (50 U ml^{-1}) or CT ($50 \mu\text{g ml}^{-1}$). The first value represents the SCC immediately before addition of the toxin. For STa the second value is the peak response, which occurred within 5 min; for CT the second value was obtained at 90 min. b the corresponding responses to glucose (10^{-2} M). All agents were added to the mucosal side of the tissue.

Cl^- secretion, when stimulated by secretagogues, although the rise in SCC associated with glucose absorption was unimpaired^{2,4}. Intestinal tissue from a control group generated an increased SCC with both secretagogues and glucose.

We have now investigated the actions of two bacterial toxins that induce a secretory response in the normal intestine — *Escherichia coli* heat-stable enterotoxin (STa), which acts via cyclic GMP⁵, and cholera toxin (CT), which acts via cyclic AMP⁵. Intestinal biopsy samples were obtained from a control group of nine children undergoing investigation for chronic non-specific diarrhoea or short stature (5 tested with STa, 4 with CT) and a group of seven CF patients, comprising three children undergoing investigation for chronic diarrhoea unresponsive to an increase in pancreatic enzyme supplements (1 STa, 2 CT), two children whose biopsies were taken at operation for

Ice-layer dating of eruption at Santorini

SIR—It appears that the authors of the correspondence on the dating of the Santorini eruption¹ had no knowledge of Baillie and Munro's² important new evidence, published one week earlier. Hammer *et al.*¹ state that the main purpose of the paper³ that stimulated the correspondence was "to show that ice-core dating, radiocarbon dates and a tree-ring (frost-ring) date all point to a seventeenth century BC date for the Santorini eruption". I believe they have succeeded in this general aim, but have reservations about precisely when in that century the eruption occurred. They concluded that the ice-core date was the most reliable, because "the acidity signal in the 1645–44 BC ice layers is clearly related to a major volcanic eruption, whereas the frost damage at 1628–26 BC ... could have been caused by climatic impacts other than volcanism". The one flaw in this otherwise reasonable argument is that the accuracy and precision of ice-layer and

tree-ring dates are equated. Whatever the cause of the frost damage in the early 1620s BC, there is no doubt of its precise date (which is in fact 1627 BC, as the time scale used by LaMarche and Hirschboeck⁴ included an arbitrarily inserted year '0' at the AD/BC transition). On the other hand Hammer *et al.*^{1,3,5} acknowledge the existence of uncertainties in the dates of ice layers (including the Dye 3 core) that are large enough to encompass LaMarche and Hirschboeck's 1627 BC event within the ice-layer date range of $1644 \pm 20 \text{ BC}$.

Hence it is surprising that Hammer *et al.*¹ see any basis for differentiating between the timing of the most prominent Dye 3 acidity peak in several centuries (their 1644 BC) and one of only three notable frost-ring events reported⁴ for the first three millennia BC. Of the other two such events, one follows the documented eruption of Etna in 44 BC by a year or less and is close to the Camp Century acidity peak reported⁵ for $50 \pm 30 \text{ BC}$, and the

Greenland ice acidity peaks and Irish oak low-growth (5289–116 BC)

Acidity peaks over 4 microequivalents kg^{-1} ice*	Low-growth events†
210 or 260	207 (1-yr event)
1120	1150s (all)
1644	1620s (all)
—	2340s (4–16)
2690	—
3150	3190s (all)
4400	4370s (all)
—	5060s (4–16)

* Ice-layer dates from Fig. 3 of ref. 5, except for 1644 which is from ref. 3. Following ref. 3, 1390 BC has been omitted. Note that these may differ from other dates for the same events given elsewhere in ref. 5. The authors of refs. 3 and 5 give error limits in the range $\pm 20 \text{ yr}$ to $\pm 120 \text{ yr}$ for these dates.

† Low-growth events from ref. 2. Narrowness indices were calculated for 10-, 1-, 2-, 4-, 8- and 16-yr windows. The windows for which an event was notable are given in parentheses after the decade. Other than 207 BC only those events that are strong at several window sizes are listed.

other may be linked with a very explosive eruption of Mt St Helens, a few hundred kilometres to the north of the trees⁶.

Even though Ireland is relatively close to the Greenland icecap, it is remarkable that of the six ice-layer acidity peaks of over 4 microequivalents kg^{-1} ice reported^{3,5} for the common prehistoric period, four coincide with or are very close to the most consistent Irish low-growth events, and one of the remaining two matches the 207 BC one-year low-growth event². Only two of the seven most prominent low-growth events fail to coincide with any of the six top ice-layer acidity peaks (see table). The growth minimum of the 1620s BC is deepest in 1628 BC (M.G.L. Baillie, personal communication), only one year before the 1627 BC frost ring in California, surely evidence of a major climate event consistent with the effects of major explosive eruptions on the climates of both regions^{4,7,8}. That these events occur within two adjacent summers during the range of possible dates for the Dye 3 acidity peak and that there is such a coincidence between the major Greenland acidity peaks and Irish oak low-growth events is compelling circumstantial evidence for an early 1620s BC date for the '1644 BC'

acidity peak and hence for the eruption of Santorini.

This suggests to me that much could be gained by an integrated use of tree-ring records with several ice-layer records, from suitable low-latitude icecaps as well as polar, to develop a precise and accurate chronology of climatically effective eruptions over the last few thousand years. This would be greatly helped if it were possible to demonstrate in tree rings an elemental or isotope fingerprint of major explosive eruptions.

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Less sinister statistics from baseball records

SIR—It has been suggested^{1,2} that lateral preference ('handedness') influences life expectancy, the inference² being that left-handers really are more gauche and maladroit than their more dexterous counterparts. But a Kolmogorov–Smirnov test⁴ shows that even if there were no difference in the mortality distributions of left-handers and right-handers, a difference in the cumulative survival fraction larger than that presented by Halpern and Coren¹ would arise by chance in about 98 out of 100 samples of the size presented there. This inspired me to investigate a much larger sample.

I used the statistics on birth, death and handedness of baseball players in *The Baseball Encyclopedia*³. Following Halpern and Coren¹, handedness was

determined by both throwing and batting hand (LL and RR, where the first letter represents the batting hand, the second the throwing hand). Of 4,220 players, 645 were LL, 2,829 were RR; LRs and RLs were excluded from the sample. Eighteen per cent of the total sample (pitchers and batters born between 1842 and 1922) was left-handed. The average life expectancy was 66.7 yr for LLs and 66.8 yr for RRs.

In calculating the difference (RR minus LL) in the fraction of players surviving to a given age, I noticed that as the sample size increased, the percentage difference decreased, as expected if the difference were due only to statistical fluctuations (see figure). On the null hypothesis that the two mortality distributions are the same, a Kolmogorov–Smirnov⁴ test

shows that a difference larger than the maximum difference (0.035) between the cumulative survival fractions in our full sample would be expected with $P = 0.54$.

Because that test examines only the maximum amplitude of the differences, not their smoothness and breadth, 25 Monte Carlo simulations were performed using the full data set and a 'bootstrap' method, in which a handedness was randomly assigned (with $P = 0.18$ for LL as in the real sample) to each age at death in the real sample. In about half of these simulations difference curves were as smooth as that in the figure, but with comparable or larger amplitude. Thus the hypothesis that RRs and LLs have the same mortality distribution cannot be rejected with $P > 0.5$.

Although switch hitters are common, there is only one recorded switch thrower, so throwing hand would seem to be an extremely accurate indicator of lateral preference. I therefore repeated the tests on the sample of 3,454 Rs and 765 Ls defined by throwing hand alone; the results were not significantly different from those reported above.

I conclude that there is no statistically significant difference between mortalities of left-handed and right-handed baseball players; to uncover any real difference would require a sample containing at least 10,000 left-handers. Part ($17 \pm 2\%$) of the full sample was left-handed from age 20 to age 90. Therefore, this sample provides no support for the claim^{1,2} that the percentage of left-handers in the population declines dramatically above age 50.

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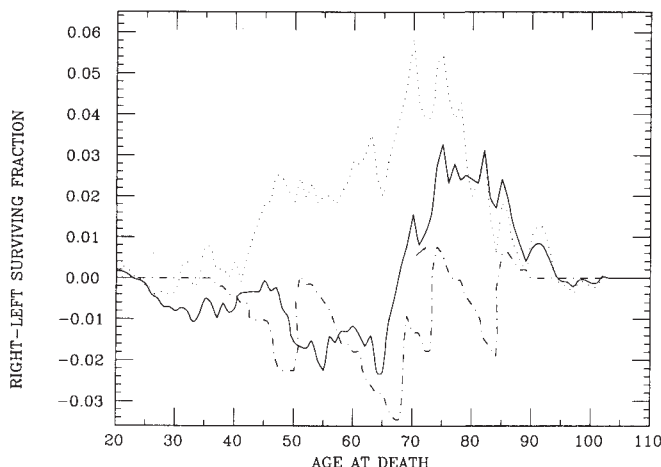
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AIDS and common sense

SIR—Those of us who are involved in the care of people with AIDS view the Byzantine 'scientific' arguments of Duesberg and of Rubin (*Nature* **334**, 201; 1988) with dismay. Common sense is obviously discarded. Rubin's closing statement, suggesting an equivalent risk of infection from transfusion (often multiple) of blood and blood products to patients, many of whom have severe existing disease, with the minute volumes transferred by needle sticks into usually healthy adults is ludicrous and calls into question any scientific merit of his arguments.

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Difference between fraction of right-handers and left-handers surviving as a function of age of three groups. Dot-dash line, group of 57 players active between 1876–1900 ($\times 0.1$); dotted line includes an additional 1938 players active between 1901–1919; solid line, total sample of 3,474 players.

The computational options

Christof Koch

Neural and Brain Modeling. By Ronald J. MacGregor. *Academic*: 1988. Pp.643. Hbk \$89, £56; pbk \$35, £22.

IT HAS become very fashionable in the past few years to investigate various aspects of the nervous system from a theoretical point of view. Indeed 'experimental neuroscience' (until recently, quite simply 'neuroscience') has acquired a twin, 'computational neuroscience'.

This new field is characterized by two generally complementary but sometimes conflicting approaches. The 'bottom-up' strategy starts with a reasonably complete description of the system under study in terms of simple biophysical or neurophysical variables and attempts to follow its evolution in response to stimuli, usually on the basis of differential equations. The most successful instance of such a model is Hodgkin and Huxley's analysis in 1952 of the initiation and propagation of action potentials in terms of time- and voltage-dependent membrane conductances. Today, the same technique — combined with the dendritic cable theory developed by W. Rall — is being applied to study the electrical properties of single identified neurons with their extended dendritic trees and dozens of membrane conductances. Similar detailed models are being elaborated at the neuronal network level, for instance in the case of a handful of neurons in the mollusc *Tritonia* or with thousands of neurons in the mammalian cerebellum or olfactory cortex.

In contrast, the 'top-down' strategy, popularized by D. Marr's influential book *Vision*, stems from the realization that it is best first to analyse complex information-processing systems, whether natural or artificial, at the abstract or 'computational' level, well divorced from the hardware. At this level of analysis, any system, whether silicon or carbon based, will deal in a similar manner with the problem at hand, for instance with the reconstruction of the third dimension (depth) from two shifted, two-dimensional images. The two systems will differ as to how this is actually achieved in practice. In recent times, the limitations of a pure bottom-up approach, as practised by most neurophysiologists, and the pure top-down approach, as advocated by researchers in artificial intelligence, have been rejected in favour of a combination of both.

Although several books have appeared over the past years epitomizing the computational approach (most recently D. Rumelhart, J. McClelland and the PDP Research Group's *Parallel Distributed Processing*), no up-to-date work extols the

virtues (and the methods) of modelling neuronal hardware at the dendrite, single neuron or neuronal network level. Thus, MacGregor's *Neural and Brain Modeling* will find an expectant audience.

The first 200 pages provide an overview of models, past and present, drawn primarily from the pages of *Biological Cybernetics* and *Journal of Theoretical Biology*. This survey includes accounts of Hodgkin and Huxley and their intellectual spawn; the application of cable theory to passive and active branched structures; models of the oculomotor system, the cerebellum, the visual, auditory and motor cortices; models of the electroencephalogram and epilepsy; models of learning and memory in neural networks; and the information-processing paradigm. There are no divas or stars for MacGregor though. The good, the bad and the ugly — everybody seems to deserve his couple of lines, with little regard to whether their idea proved to be dead on arrival or resulted in a worthwhile advance in our understanding, and the models are always "powerful", "coherent", "elegant" and "exciting". Some theories, long out of fashion with both the neurobiological and the modelling community, are given plenty of space, for instance the fluid mechanical models of Beurle in the 1950s and of Griffith in the 1960s in which neural

networks were studied by formulating differential equations representing spatio-temporal spread of neuroelectric activity. Other models receive scant mention, as for instance W. Reichardt's integrative approach towards understanding the visual system of the flies at the cellular, system and theoretical levels.

Ultimately, the usefulness of any abstract, mathematical model of reality has to be judged by its explanatory and predictive power. Specifically, what impact did the neural and brain models discussed in the book have on biophysics, neurophysiology or psychophysics? What is the relevance of these theories to experimental neuroscience? Fatally, MacGregor avoids these issues.

The second part of the book is organized around a number of annotated FORTRAN programs (including 180 pages of code; but at least we are spared flow charts), together with instructions, examples and exercises, enabling the user to model a large variety of neuronal systems, from a single-node neuron, to more sophisticated models with dendritic trees and active conductances, to systems of neuronal populations. MacGregor even includes a program to study the propagation of action potentials in branched axonal trees, an important and almost untouched area of research. This collection clearly represents the fruits of many years of effort. But although the range of phenomena which can be simulated numerically is impressive, the execution of these simulations is less so. For instance, little space is given to the seminal issue of the proper numerical technique to use for solving particular types of differential equations. The use of parallel



"Physicists are often accomplished musicians but, surprisingly, excellence in physics is rarely matched with creative ability in the visual arts" — so runs the jacket blurb to *The Physicist as Artist: The Landscapes of Pierre Duhem, selected and introduced by Stanley L. Jaki*, from which this detail of *En Camargue (1899)* is taken. Duhem, who died in 1916, was a prominent theoretical physicist, philosopher and historian of science, and produced over 400 landscapes many of which are reproduced in the book. Publisher is Scottish Academic Press, price is £25.

(concurrent) computers to simulate inherent parallel structures such as the brain is dismissed in a mere five lines.

Furthermore, questions of the proper input and output routines crucial to any large-scale simulations of neuronal structures are not even raised. Anybody who has attempted to write a general simulation package for neuronal models has quickly come to realize that a high-level, symbolic language to define and specify channels, dendrites and neuronal types (for example, pyramidal versus stellate cells) is needed. In that manner, very complex neurons or neuronal populations can be constructed using simple elements. Sophisticated programs even use graphical icons (little synapses and cables, for instance) in conjunction with a pointing device such as a mouse to achieve this. The question of how the activity state of thousands of neuronal elements can best be evaluated and displayed must also be addressed (a problem which is shared by experimentalists using multi-electrode

arrays to record from an ever larger number of neurons). Such apparent 'frills' dramatically reduce the time spent writing, debugging and understanding large programs. But for the most part these problems are not easily dealt with in FORTRAN, which lacks such features as dynamic data structures and recursion.

As a methodological handbook, *Neural and Brain Modeling* is not a success. It could serve as a sort of *Who's Who* in modelling, although the references are scattered, by year of publication, in over 60 separate bibliographies spread throughout the book. But the novice modeller would do better to read J. Jack, D. Noble and R. Tsien's seminal *Electric Current Flow in Excitable Cells* (now in its second edition) together with a standard book on numerical techniques, and to start programming in C. □

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Three down . . .

C. M. Perrins

The Birds of Africa, Vol. III. Edited by C. Hilary Fry, Stuart Keith and Emil K. Urban. Academic:1988. Pp. 611. £71.50, \$129.

The Birds of Africa continues to grow. When the first volume appeared, the plan was to cover all the species in four volumes; those who added up the birds dealt with in that volume and looked at the number left were puzzled as to how this was to be achieved. By Vol. II it was conceded that six volumes would be needed; now, in Vol. III, we read that seven will be necessary!

Apart from the fact that it will take longer to complete, and will cost more, there is everything to be gained from this increase in volumes. Knowledge about African birds has rapidly expanded since 1982, when the first volume appeared, and the amount of text space per species is not excessive — some 311 are covered in 556 pages, a little under two pages per species, and this includes a range map which takes up about one-sixth of a page.

This volume completes the non-passerines, an uncomfortable term describing all the birds that do not belong to the enormous Order Passeriformes, or songbirds. Better known to most people will be many of the groups covered: parrots, turacos, cuckoos, owls, nightjars, swifts, mousebirds, trogons, kingfishers, bee-eaters, rollers, hoopoes, woodhoopoes, barbets, honeyguides and woodpeckers. These birds differ from those described in Vols I and II in that many of them are not



Red and yellow bar-bets, colourful in life and in Martin Woodcock's illustration in *The Birds of Africa* — spotted and blotched red, yellow, black and white, with diagnostic long reddish bill and red, white and black ear patch — here shown at about half the size of Woodcock's painting.

migrants, and so never leave Africa. They will thus be familiar only to those who live or travel in the continent. Indeed several of the families are ones that are largely confined to the tropics.

Special mention must be made of the mousebirds (Coliiformes), an odd little order containing just six species; this is the only bird family wholly endemic to Africa and seems to have remained unchanged through the ages. Fossils from 22 million years ago are almost indistinguishable from their living descendants, and recent work using DNA-DNA hybridization indicates that they may have separated from other birds about 100 million years ago (*Archaeopteryx*, the first bird, only appeared about 150 million years ago).

After many years of comparative stability, major changes in bird classification are on their way as a result of new methods of establishing relationships, particularly

the DNA-DNA hybridization techniques pioneered by Sibley and Ahlquist. By no means all the proposed changes are yet accepted, so that it is difficult for editors of a work such as this to know how to respond. They have taken what is, to me, a sensible course, especially considering that they are now well into the series; they have mentioned the changes that have been proposed for the birds in this volume, but have retained the standard, old structure, warts and all. This may be more difficult in future volumes as the new ideas become better established (which many of them surely will).

The layout of this volume is similar to that of the previous ones, and the same subjects are covered: range and status (including migratory habits where relevant), description, field characters, voice, general habits, food and breeding habits. Inevitably, for some birds, especially difficult ones such as swifts and forest dwellers such as owls and turacos, little is known; in some cases no nest has ever been recorded. But what we do know is brought together most usefully in this volume.

As in Vols I and II all the plates have been painted by Martin Woodcock. To say that he improves with time might be taken as a criticism of the earlier paintings, but the colour plates in the current work are very pleasing indeed. It has been necessary to cover many species on each of the 32 plates, but the layout has been effectively constructed. For example, on one of the two plates of nightjars 16 birds are depicted in detail and another 19 shown, to a smaller scale, in flight. Yet I do not find the plate uncomfortably cramped. The publisher and printer also need some compliments for the colour reproduction; for example Plate 3 (of turacos) incorporates many shades of grey and most colours of the rainbow, but is well-balanced over the whole range of them. The names of the birds and cross-references to the relevant text pages are on the opposite pages to the plates. A nice touch which I do not remember having seen in other works is that all birds of the same species (different sexes or sometimes different subspecies) are pulled together by overlaying their names with a grey tint, thus usefully drawing one's eye to this fact. Mention must also be made of the many helpful line drawings in the text by Ian Willis.

So, *The Birds of Africa* continues on its stately publication schedule, on its way to becoming the standard work on African ornithology for the twenty-first century. The next volume is due in 1990, which means that at the current rate of progress ornithologists may have something special to celebrate in the year 1996. □

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Civilized origins

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Peruvian Prehistory. Edited by Richard W. Keatinge. *Cambridge University Press*: 1988. Pp.364. Hbk £35, \$49.50; pbk £13.50, \$15.95.

DOMINATED by the Andes, and flanked by the Pacific Ocean to the west and the Amazon rainforest to the east, Peru is a land of geographical extremes with an equally diverse and rich archaeological heritage. The accelerating pace of multi-disciplinary research over the past 30 years has resulted in a great increase in our knowledge and understanding of the region's prehistory, making a single-volume appraisal a difficult but worthwhile undertaking.

The book is organized chronologically, and the bulk of contributions deal with three main themes — Peru's early inhabitants; the development of complex societies; and the origins and spread of the pan-Andean Inca empire. Given the paucity and ambiguity of evidence before about 11,000 BC, the problem of dating America's first inhabitants has often degenerated into sterile argument between personalities. By concentrating on the better-known post-glacial Pre-ceramic period, between c. 9500 and c. 1800 BC, both Rick (the Andes) and Chauchat (the coast) avoid this pitfall to provide critical reassessments of previous, often confusing, views.

In the Andes, human occupation on a large scale was underway by about 8000 BC; in the high puna grassland, the evidence of lithic technology, available resources, seasonal mobility and the rock-art tradition all indicate that the inhabitants were hunter-gatherers. On the coast, at a time when both sea level and coastline were markedly different from today, a distinctive physical type of human population, the *Paijanense*, possessed tools and followed subsistence strategies based on the exploitation of maritime resources.

Despite the crucial importance of this sketchily known period, much more research and argument has centred around defining the roots of ancient Peruvian civilization. For decades the prevailing dogma has been that the famous Andean cult centre of Chavín de Huántar was the epicentre from which a feline cult swept through Peru between c. 1200 and 400 BC. Today the weight of evidence sees the origins of complex society as having a much earlier, coastal origin — during the Late Pre-ceramic and Initial phases, between c. 3500 and 800 BC. In her account, Fung Pineda shows that by c. 2800 BC centres such as Rio Seco and Aspero possessed an impressive array of

monumental architecture in the form of terraces, pyramids and residential areas. In her view, such sites were unequivocally the product of a politico-religious system controlled by a priesthood, millennia before the rise of Chavín. In his chapter on the Early Horizon (c. 800 to 200 BC), Burger drastically re-evaluates Chavín — he sees it as a significant but short-lived phenomenon, during which technologically precocious innovations in metallurgy and textile production made their first appearance.

From these beginnings, some of Peru's most striking civilizations evolved on the coast between 300 BC and AD 600. In a cursory review of this Early Intermediate period, Conklin and Moseley refer to the strong regional differentiation of the era, to the limiting bias of knowledge in favour of the iconography of such cultures as Moche and Nasca, and to the gap in our understanding of the cultural processes involved.

Better documented, if somewhat dense in style, is Isbell's account of the Middle Horizon (c. AD 600 to c. 1000) from the perspective of the great highland site of Huari. This prehistoric city of perhaps 20,000 inhabitants maintained a little-understood relationship with the great ceremonial centre of Tiwanaku on the shore of Lake Titicaca in Bolivia, and played a central role in the evolution of Andean civilization. Between its demise and the rise of the imperial Inca state, the

Chimú empire flourished on the north coast. Dominated by the metropolis of Chan Chan, the paramount concern of its bureaucracy was the control of hydraulic agriculture which gave Chimú society its characteristically rigid form and which culminated in the construction of the 84-km-long Chicama-Moche canal.

The increasingly thorough use of ethnohistorical data to test and complement archaeology is particularly well dealt with in the chapters by Morris and Netherly. Careful study of written records has helped provide a provincial view of the Inca state, has elucidated the nature of intra-valley relationships on the coast (often determined by competition for the coca fields) and has yielded valuable insights into the vertical economy of the Andes.

In *Peruvian Prehistory* we have the first collective assessment of the area's archaeology for a generation. The book is at times uneven in style and coverage, and thus less accessible than one might have hoped, and some of the views expressed have changed or have been superseded. Nevertheless, the authoritative and well-referenced contributions, together with clear maps and a reasonable price, make this a valuable and welcome publication. □

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Chance encounter

George Greenstein

Not by Design: The Origin of the Universe. By Victor J. Stenger. *Prometheus*: 1988. Pp.202. \$22.95, £16.95.

MOST of *Not by Design* is taken up with a whistle-stop tour through modern physics and cosmology, with a mild emphasis on recent theories of the early Universe. The scope of topics covered is wide, ranging from thermodynamics to atomic theory to elementary particle physics to cosmology. According to the author, the central thesis underlying this survey is that the design we have found in the cosmos in no way implies the existence of a designer (or, if one prefers, a Designer). No cosmic legislature ever promulgated the laws by which the Universe operates — its order, Stenger argues, has come about entirely by chance.

This is an important thesis, and one worth exploring. Unfortunately, however, the treatment Stenger affords it is far too brief, and although his account is provocative, and in places intriguing, it fails to touch upon many of the various ramifications of his thesis. Indeed, it is

only in the first and last chapters of the book that any real attention is given to the question. Only the briefest mention is made of self-organizing systems such as crystals, snowflakes and the like, and the manner in which order appears in the physical world. Nor is there any serious treatment of darwinian evolution, natural theology and the debate over the nature of order in the biological world that historically was of such immense significance.

The survey material, too, is inadequate. To my eye, at least, most of it is entirely unrelated to Stenger's central thesis, and would have stood quite well on its own. Furthermore, because the range of topics covered is so very wide, the coverage of each of them is correspondingly brief — Bohr theory is covered in two paragraphs, black holes in a page, Maxwell's unification of electricity and magnetism in three pages. The book is clearly intended for the general reader, but on occasion the discussion becomes so technical as to be comprehensible only to the initiate.

Certain errors and oversimplifications have crept in, both of a historical and a factual nature. For example, Stenger invokes Occam's Razor in relation to the copernican world-system, claiming that Copernicus required fewer epicycles, eccentrics and the like than Ptolemy to

account for the motions of the planets. In fact he did not, and the historical reasons for the acceptance of the heliocentric cosmology are far more complex. Osiander's notorious preface to *De revolutionibus*, which was almost certainly included without Copernicus's knowledge or approval, is quoted as representing Copernicus's own beliefs. (Osiander argued that the success of a theory in accounting for a series of observations in no way implies the truth of that theory. The argument is quoted by Stenger with approval, who claims that it represents the position of all modern scientists with respect to their work — a claim that many of them would dispute.) The Big Bang is repeatedly referred to as an explosion in which the material of the cosmos, initially concentrated at a central point, expands into empty space; the non-euclidian elements of relativistic cosmology are entirely ignored. And the gravitational deflection of light is presented as being purely special-relativistic in nature, no mention being made of the well-known factor-of-two error by which this attempt at an explanation fails.

Not by Design is a splendid title. It is a pity that Stenger has failed to write a book that lives up to it. □

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Green genes

Ian D. Small & C. J. Leaver

Plant Genetic Transformation and Gene Expression: A Laboratory Manual. Edited by John Draper, Roderick Scott, Philip Armitage and Richard Walden. *Blackwell Scientific*: 1988. Spiral bound pp. 355. £29.50, \$66.50.

Plant Molecular Biology Manual. Edited by Stanton B. Gelvin and Robert A. Schilperoort. *Kluwer*: 1988. Loose-leaf core manual pp.400. Dfl. 110, £31.50, \$52.50.

EVERY laboratory has a set of protocols which evolve on scraps of paper, annotated with the latest modifications, ready for production as THE LAB MANUAL. Few such manuals make it to the presses, and fewer still become classics. But in an expanding field like plant molecular biology the opportunity is there for a beginner's practical manual to make a big impact, and the candidates are appearing thick and fast.

Although there is no substitute for hands-on experience, *Plant Genetic Transformation and Gene Expression* is the next best thing. Based on a course run at Leicester University, it is clearly written, well laid out, helpfully illustrated and covers the basics of the field comprehensively. Its six chapters deal with vectors for gene transfer, plant transformation using *Agrobacterium* (the largest chapter), direct DNA uptake into protoplasts (with or without electroporation), isolation of plant nucleic acids, Southern blot analysis of plant nuclear DNA and the analysis of gene expression in transgenic plants. A welcome extra is a list of addresses of companies cited in the text as suppliers of equipment and materials, although it will be of more use to readers in Europe than those elsewhere. All in all, the book has been thoroughly designed to enable a laboratory with little previous experience of molecular biology or plant tissue culture to start work with these techniques.

Plant Molecular Biology Manual comes in a loose-leaf binder, and providing you have the funds to buy them, and the editors keep their promise, updates will become available as the techniques change and new technologies are devised. The manual is divided into three sections: introduction of DNA into plant cells (nine chapters), expression of genes in plants (seven chapters) and the fate of introduced genes (two chapters). Each of the chapters is self-contained and is written by specialists, who, in most cases, have considerable expertise on the particular facet of plant molecular biology involved.

This type of book has its disadvantages, and in comparison with *Plant Genetic Transformation* it suffers from a lack of

continuity and cohesion; for example, different protocols for the same technique appear in different chapters (with no cross-referencing). Unlike the other book, there is no index, so hunting for a particular technique (with no way of knowing whether it is included) can be frustrating. The compensating advantage is the wider scope achieved by having so many contributors, which allows the inclusion of several additional subjects, among them the construction of cDNA and genomic clone banks, chromatin structure, DNA methylation, translation of *in vitro* generated mRNA in *Xenopus* oocytes and RFLP mapping.

Where there is duplication between the two manuals, *Plant Genetic Transformation* is more comprehensive, and offers more practical advice to beginners. Protocols often differ slightly between the two books due to the use of different plant material. Plant cells and tissue taken from different species, or from the same species grown under different conditions, or even from different parts of the same plant, often require modifications of the same technique. Draper and his colleagues attempt to get round this problem by describing experiments to optimize new systems in addition to giving protocols for the systems they use. Gelvin and Schilperoort confine themselves to providing references for protocols with other known systems. Both books appear reasonably up to date, although the omission of a description of the promising new reporter gene β -glucuronidase (GUS) in either of *Plant Molecular Biology Manual's* two chapters on reporter genes is unfortunate.

For the worker new to the field, *Plant Genetic Transformation* is ideal. Groups with a specific idea of the plant system and techniques they wish to use should check which book has better coverage for their purposes, while researchers who already have a working transformation system may prefer the greater variety of techniques in *Plant Molecular Biology Manual*. Preferably, of course, one should have both! □

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• With two new spiral-bound ('bench-top format') books on its list, Academic Press is also a player in the growth business of publishing practical manuals in plant molecular biology.

Methods for Plant Molecular Biology, edited by Arthur Weissbach and Herbert Weissbach, is aimed at graduate students and more experienced researchers, and consists of articles chosen from Vol. 118 of the series *Methods in Enzymology*. Price is \$45, £31.50. Mary A. Schuler and Raymond E. Zielinski's *Methods in Plant Molecular Biology* is due to appear later this year, and is an introduction to various techniques primarily intended for students. Price is \$29.95, £19.

A new view of the mid-ocean ridge from the behaviour of ridge-axis discontinuities

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High-resolution images covering large areas of the seafloor reveal numerous discontinuities along the mid-ocean ridge. These discontinuities occur at a range of scales (10–1,000 km) and define a fundamental segmentation of seafloor spreading centres. Some are transient; others persist for millions of years, migrating along the mid-ocean ridge and disrupting the structural and geochemical character of approximately 20% of the oceanic lithosphere.

THE mid-ocean ridge is perhaps the most significant geological feature on the Earth, and in the plate-tectonic model it is a constructive plate boundary (the 'spreading centre'), along which most of the Earth's rigid outer shell (or 'lithosphere') is created. In this context the mid-ocean ridge is seen as a continuous feature, interrupted only by lateral offsets of the spreading centre along transform faults, where lithosphere is neither created nor destroyed as the rigid plates slide past each other^{1,2}. During the 1960s and 1970s our understanding of the shape and structure of the ridge system was based on widely spaced profiles which had limited resolution and were oriented perpendicular to major structures. These data led to a useful but oversimplified view of mid-ocean ridges as simple, linear, two-dimensional structures.

Problems with this view became apparent when high-resolution 'swath'-mapping systems provided a new generation of bathymetric data for mid-ocean ridges. With these systems, a swath of sea floor several kilometres to tens of kilometres wide can be mapped on a single pass, at moderate (100 m) to very high (1 m) resolution^{3,4}. The resulting combination of adequate resolution and continuous coverage over thousands of square kilometres has revealed that variations in lithospheric structure parallel to the strike (or trend) of mid-ocean ridges are at least as significant as variations across strike. On a large scale, the two most important variations observed along the strike of the ridge axis are the smooth but continuous change in the depth of the axis of the spreading centre, and the occurrence of non-transform ridge-axis discontinuities such as propagating rifts and overlapping spreading centres at local depth maxima in the axial depth profile^{5–7} (see Fig. 1). These discontinuities, as well as transform faults, partition the spreading centre into segments which may migrate, and may also lengthen or shorten with time^{8–10}. The amount of magma available at each segment and the composition of erupted lavas, as well as the timing and intensity of tectonic activity, may vary in a systematic way along strike within each segment.

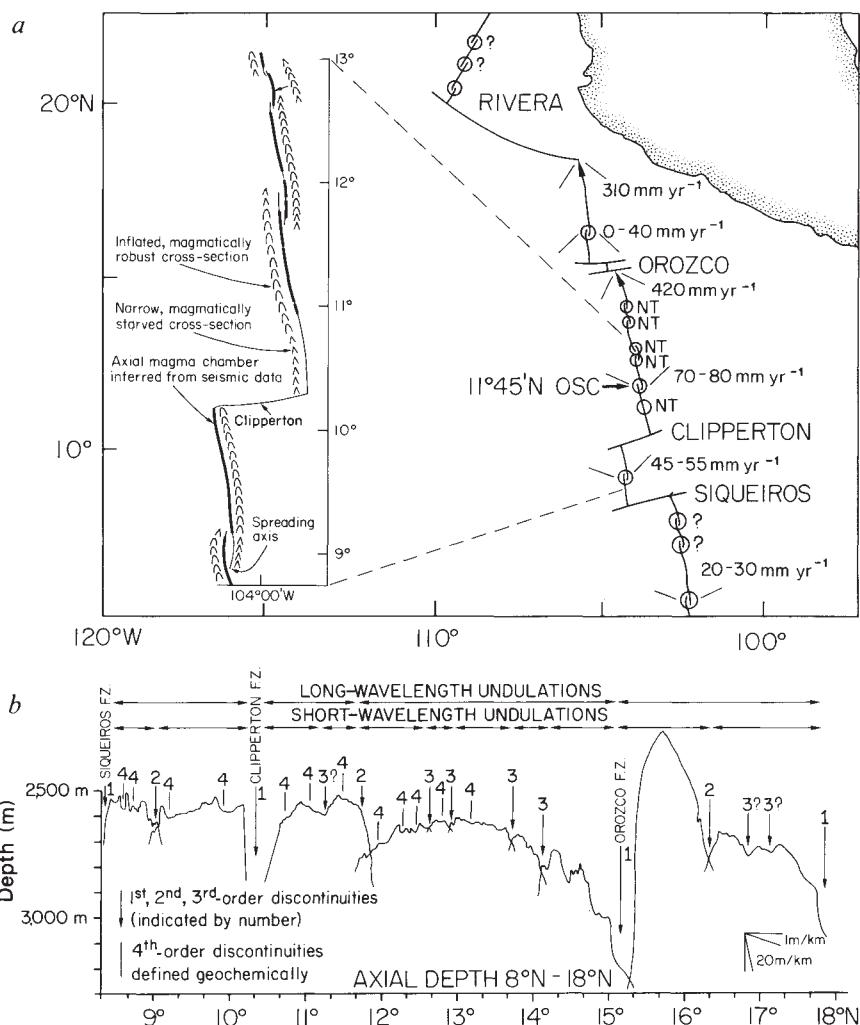
Characteristics of segmentation

Transform faults are rigid plate boundaries where the ridge is offset, juxtaposing old lithosphere of varying age and thickness against the ridge axis. These boundaries, which typically have offsets of 50 km or more, partition the ridge into distinctive tectonic units which persist for millions of years or more. For example, the Clipperton transform along the East Pacific Rise (EPR) has persisted in its present configuration for 9 Myr¹¹. It offsets the ridge by 85 km (an age contrast of 1.5 Myr across the

transform), and separates two ridge segments with contrasting tectonic and petrological properties^{12,13} (Fig. 2). The northern spreading segment deepens and narrows over a long distance (~60 km) towards the transform fault, whereas the southern segment remains shallow and wide all the way into the western ridge-transform intersection (see Fig. 2). In addition, the composition of the basalts comprising the ocean crust is very different north and south of Clipperton, and suggests that the lavas were erupted at lower temperatures on the northern segment than on the southern segment^{14,15}. A detailed multi-channel seismic reflection experiment along the EPR from 8° to 13° N shows a bright reflector at ~1.5 km depth, which is interpreted to be the top of an axial magma reservoir¹⁶. This reflector continues all the way into the Clipperton transform from the south, whereas to the north there is a gap of 70 km in the axial magma chamber¹⁶ (Fig. 1). Thus, nearly every measurable parameter related to shallow structure and local magmatic budget is highly asymmetric across the Clipperton transform, consistent with the idea that these two segments are evolving independently; the segment north of Clipperton is relatively cool and starved of magma, whereas the segment south of the transform is robust and swollen with magma¹⁷. These differences in segment character cannot be explained by the thermal edge effects introduced by placing old, colder lithosphere against a ridge axis, because these effects should be the same on both the northern and southern ridge segments.

Overlapping spreading centres. Between the widely spaced transforms along the EPR, the axial continuity of the ridge is frequently interrupted by a new class of sea-floor structure, a non-rigid discontinuity called an overlapping spreading centre (OSC)⁵. Recent swath-mapping of the EPR has revealed that at OSCs the spreading centres are offset by a short distance (0.5–10 km), and overlap each other by approximately three times the offset distance^{6,7}. The offsets at OSCs are far too small for there to be any measurable thermal edge effect, as the lithosphere juxtaposed against the spreading centre at an OSC is still thin and hot (generally less than 5 km thick and 50,000–200,000 years old)^{6,38}. Yet there are systematic and significant along-strike changes in the spreading-centre axial depth profile, the geochemistry of erupted lavas, the fine-scale structure of the ridge and the magma-chamber reflector near OSCs (Fig. 1). The 11°45' N OSC, which offsets the EPR by only 8 km, is an excellent example^{17–19}. The axial depth plunges several hundred metres over tens of kilometres near this small discontinuity, and there is a significant change between the regional depths of the segments north and south of the OSC (Figs 1b and 3). Given that

Fig. 1 Section of the East Pacific Rise (EPR) mapped by SeaMARC II and Sea Beam sonar systems. *a*, Map view of plate boundaries, showing segmentation of the ridge by transform faults (marked "Siqueiros", "Clipperton", etc.) and overlapping spreading centres (circled). Speeds in mm yr^{-1} are the migration rates of the discontinuities along the ridge axis (this study and refs 6, 35, 36). Question marks indicate inadequate data on flanks; NT, adequate data but no off-axis trace. Arrowheads indicate two propagating rifts which have recently propagated north into fracture zones. At left is an enlargement of the section from 9° to 13° N, showing the structure of the ridge and the occurrence of an 'axial magma chamber' reflector detected during a multi-channel seismic experiment¹⁶. Where there is an axial magma chamber reflector, the cross-sectional shape of the rise tends to be broad and an axial summit graben is usually present, and this occurs along shallow parts of a given segment away from discontinuities¹⁷. Where the axial magma chamber reflector is absent, the ridge is narrow (triangular symbol) and deep, and a summit graben is missing; these characteristics occur near first-, second- and third-order discontinuities (see text and Fig. 4 legend for discussion). The depth, cross-sectional shape and fine-scale structure of the rise reflect along-strike variations in the magmatic budget associated with magmatic segmentation of the ridge. Fourth-order discontinuities are not shown. *b*, Axial depth profile of the EPR from 8° to 18° N, extracted from Sea Beam records. Discontinuities of order 1–3 (transforms and overlapping spreading centres (OSCs), see text) are identified from Sea Beam charts^{6,7}; those of order 4 are identified from geochemical data between 8° and 13° N (the data are inadequate north of 13° N)¹⁴. Long-wavelength undulations in the axial depth profile are punctuated by first- and sometimes second-order discontinuities, whereas short-wavelength undulations are marked by third-order discontinuities. Fourth-order discontinuities have little or no bathymetric signature. Note how the rise segment north of the Clipperton transform, which has a small triangular cross-section (see *a*), is deeper (on average) and plunges over a longer distance towards the transform than does the ridge segment south of Clipperton, which has a sustained, broad cross-section. Also note the major depth anomalies at second-order discontinuities, especially the OSC near $11^\circ 45'$ N. ("F.Z." stands for fracture zone—the geomorphological expression of a transform fault.)



the offset at the $11^\circ 45'$ N OSC is so small, the axial magmatic and thermal budget must be controlled by processes at depth beneath each of the spreading segments, rather than by the near-surface boundary conditions imposed by small offsets in lithospheric plates.

Magmatic budgets. A clue to the processes controlling segmentation comes from a comparison between multi-channel seismic data¹⁶ and swath bathymetric data for the EPR from 9° to 13° N¹⁷. There is an excellent correlation between three parameters which are all directly related to the magmatic budget along a given ridge segment: the cross-sectional shape of the rise, the presence or absence of an axial summit graben, and the presence or absence of a shallow axial magma chamber, as interpreted from seismic data (see Fig. 1). Where the axial magma chamber is present, the cross-sectional shape of the ridge is broad and an axial summit graben is recognized along the axis. In contrast, where the cross-sectional shape of the rise is narrow and triangular, an axial magma chamber is not detected and an axial summit graben is absent. These latter characteristics tend to occur along deeper portions of a given ridge segment, often near ridge-axis discontinuities. These systematic variations in morphology (cross-sectional shape) and structure (presence or absence of an axial graben) may reflect spatial and temporal variations in the magmatic budget of the ridge axis¹⁷. Where

the supply of magma is waxing, shallow-level magma reservoirs in the crust and underlying upper mantle swell, creating a broad axial bulge with a summit graben. Where the magma supply is diminished, the crustal magma chamber is small ($<2 \text{ km}$ wide) or absent and the ridge axis is characterized by a narrow triangular edifice which lacks a clearly defined and continuous axial graben. A summit graben is missing because the underlying axial magma reservoir is not large enough to produce a significant collapse structure or caldera¹⁷.

Thus, a new view of the mid-ocean ridge is emerging, a view in which the ridge is partitioned into segments which are defined by the distribution of sources of partial melt at discrete locations beneath the ridge axis^{6,9,10,12,20,21}. Major episodes of decompression partial melting are triggered $\sim 30-60 \text{ km}$ beneath the ridge axis as the plates spread apart²². Partial melt beneath the ridge axis segregates and ascends, feeding axial reservoirs at discrete places along the plate boundary—for example, at the shallow areas north and south of the $11^\circ 45'$ N OSC as well as on either side of the Clipperton transform¹⁷⁻¹⁹ (Fig. 4). These melts locally swell the crustal magma reservoirs, and the buoyant forces associated with the melt and surrounding halo of hot, low-density rock create a local high in the axial depth profile of the ridge²³. Continued injection of melt leads to local eruptions, as well as migration of magma away from the source of

upwelling, swelling the axial magma chamber along strike. The laterally migrating magma loses hydraulic head with increasing distance from the centre of magma replenishment, creating a graded axial depth profile along strike. As magma migrates along the axial accretionary zone, the overlying brittle carapace stretches and fractures; magmas then use these fractures as conduits to the surface and volcanic eruptions follow in the wake of an advancing, cracking front. Shortly after an eruption, the reduction in volume of magma leads to removal of support and a resulting collapse of the sea floor along the axis, creating the axial summit graben. In this magmatic model for the spreading centre, ridge-axis discontinuities occur at the distal ends of magmatic pulses, usually at deep points along the axial depth profile, and define the ends of ridge segments (Fig. 4). This model implies some degree of active mantle upwelling on short temporal and spatial scales ($\sim 1\text{--}50\text{ km}^2$, $10^2\text{--}10^4\text{ yr}^2$), against a background of passive rifting and passive mantle upwelling on greater temporal and spatial scales.

Scales of segmentation

The segmentation of the ridge is observed at a range of scales (Fig. 4). The first-order segmentation of the ridge is tectonically defined by major transform faults and propagating rifts, whose offsets are large enough that the lithosphere along the plate boundary behaves rigidly ($0.5\text{--}1.0\text{ Myr old}$)^{1,8}. (Propagating rifts occur where one ridge segment (the propagating rift) overruns a transform fault and lengthens along strike, as the adjacent ridge segment (the dying rift) becomes inactive⁸.) These discontinuities typically partition the ridge at intervals of 300–500 km (total range of 100–1,000 km) and leave a clear signal of offset magnetic anomalies on the sea floor. They are characterized by large axial depth anomalies of 500–3,000 m, and often by pronounced geochemical anomalies (such as fractionated basalts high in iron and titanium)²⁴.

A second-order segmentation, on a length scale of 50–300 km, is defined by smaller offsets of the spreading centre which behave non-rigidly (see Fig. 3). These include large-offset ($>3\text{--}5\text{ km}$) OSCs at intermediate-rate to fast spreading centres ($>50\text{ mm yr}^{-1}$ total opening rate) and small-offset ($<20\text{ km}$), non-rigid transform faults (where the axial rift valleys may overlap slightly) at slow spreading centres. Second-order discontinuities typically have axial depth anomalies of several hundred metres⁶. A third-order segmentation, on a length scale of 30–100 km, is defined by small-offset (0.5 km to $3\text{--}5\text{ km}$) OSCs at fast to intermediate-rate spreading centres. Axial depths are only tens of metres greater at these discontinuities than at the shallower mid-sections of intervening segments.

On an even shorter length scale of 10–50 km, fourth-order discontinuities are often characterized by very small lateral offsets ($<0.5\text{ km}$) of the axial summit graben ('small non-overlapping offsets')²⁵, and by small bends or changes in strike ($1\text{--}5^\circ$) of the axis ('deviations in axial linearity')¹⁴. These features are rarely associated with detectable axial depth anomalies (Fig. 1). At slow spreading centres, third- and fourth-order discontinuities are simply gaps in the axial neovolcanic zone between individual volcanoes within the rift valley floor. These volcanoes are typically 2–20 km long and 1–4 km wide.

If the model of magmatic segmentation outlined in the preceding section is correct, these contrasting bathymetric signatures should be associated with geophysical and geochemical differences as well. Indeed, first-, second- and third-order partitioning of the ridge is associated with a disruption of the axial reflector¹⁶ and distinctive geochemical characteristics^{14,19}. These data imply that the magmas from neighbouring segments have distinct mantle sources as well as different histories of melt segregation and fractionation. In contrast, the fourth-order segmentation of the ridge is usually inferred geochemically¹⁴, and only occasionally has a clear tectonic and seismic signature—one which requires very high resolution to detect (Fig. 5). Perhaps fourth-order segmentation is related to the length scale over which a

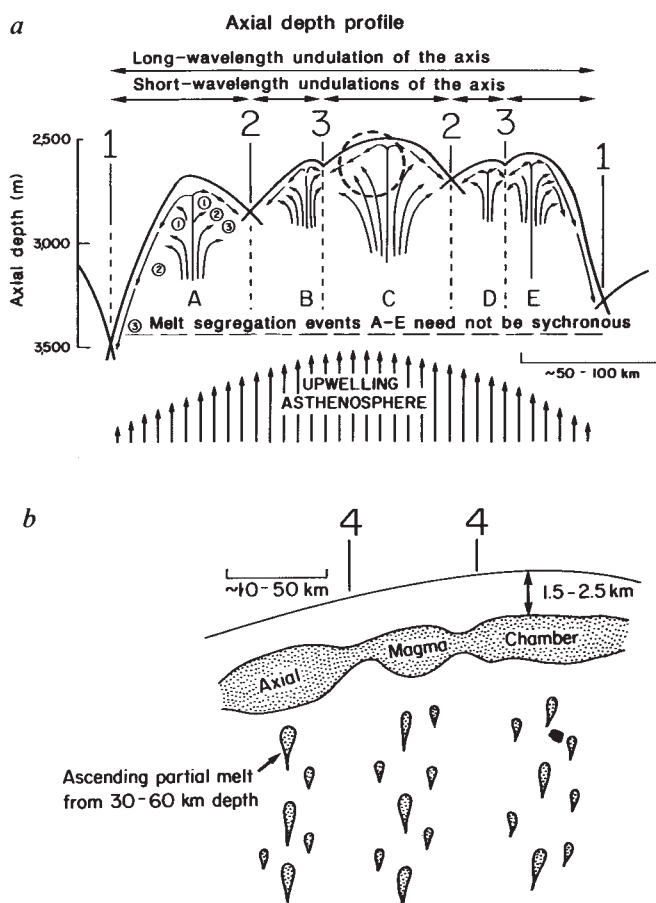


Fig. 4 Proposed model of magmatic segmentation for the mid-ocean ridge. *a*, Schematic along-strike cross-section of the ridge. First (transforms, propagating rifts), second (large OSCs) and third (small OSCs) -order discontinuities occur at local maxima in the depth profile and are indicated by large numbers (deepest, longest-wavelength maxima at first order; smallest-wavelength maxima at third order); dashed circle shows area that is enlarged in *b*, showing a possible configuration for fourth-order segmentation (see text for explanation). Although the pattern of asthenospheric upwelling is unknown, we suggest (arrows) that the source of the upwelling is enhanced beneath the shallowest portions of the ridge, but has some flux along most of the length of the ridge. Decompression melting during upwelling leads to melt segregation events (A-E), which occur 30–60 km beneath the axis²². These events replenish and inflate the axial magma chambers beneath separate segments of the ridge as shown.

small radius ($\sim 1\text{--}2\text{ km}$), if the average residence time for magma viscous magma can mix in a long chamber ($\sim 20\text{--}100\text{ km}$) of in the axial chamber is short²⁶ (Fig. 4). Local constrictions and collapses may further impede mixing, so that a single chamber, receiving contributions from more than one mantle source, may keep these magmas separate from one another up to the time of eruption¹⁷. The nature and significance of fourth-order segmentation is an important area of continued research and controversy.

Temporal evolution and kinematics

To establish the behaviour of non-rigid ridge-axis discontinuities through time, it is necessary to map the off-axis structural patterns that arise from them. With a swath width of 10 km and towing speed of 8 knots, SeaMARC II is the ideal tool³. Bathymetry can be contoured to 50 m routinely, and fine-scale structures such as fault scarps are detectable to heights of several metres and lengths of 100–300 m on the side-scan record²⁷. In 1987 we conducted two month-long expeditions to image the

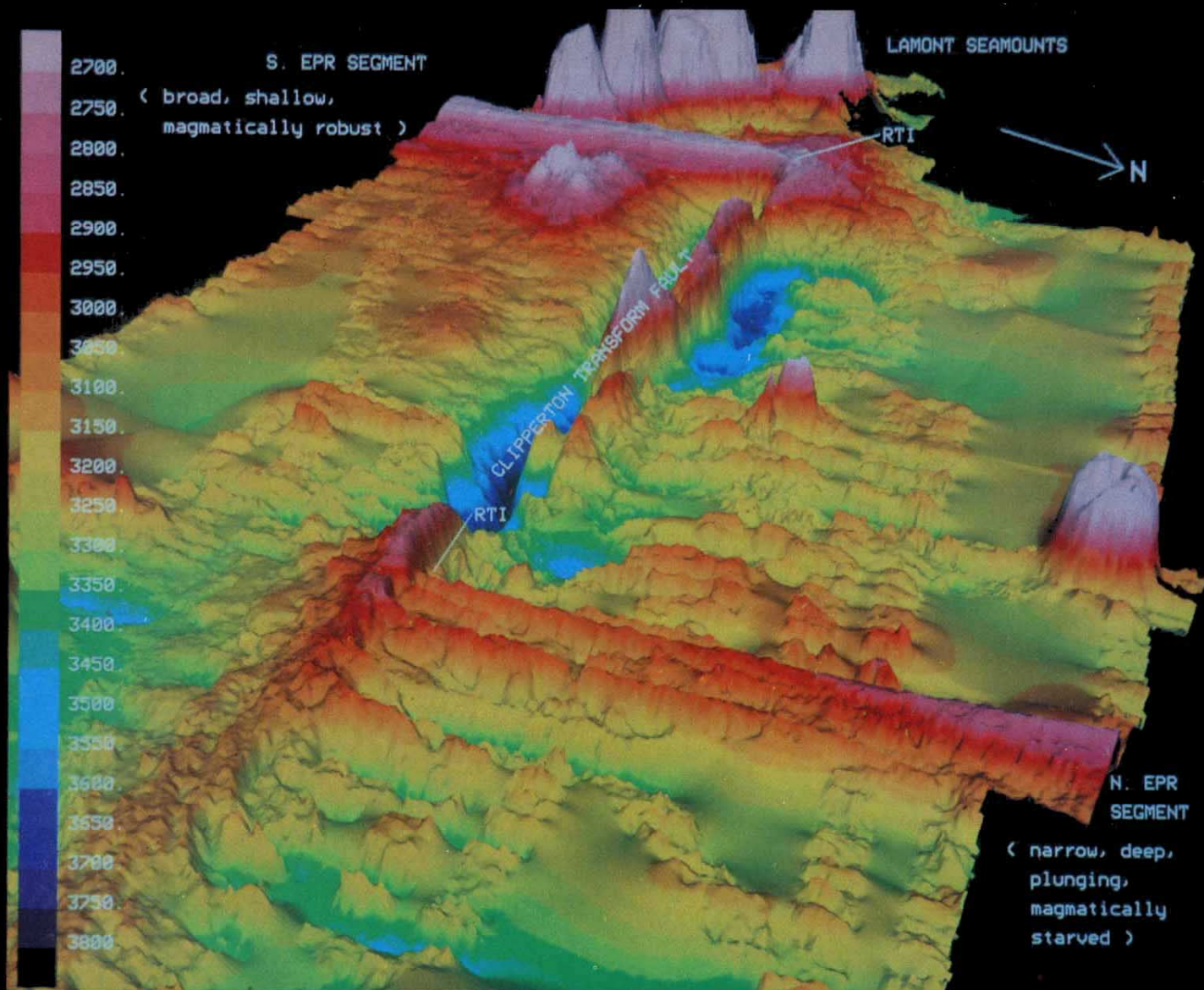
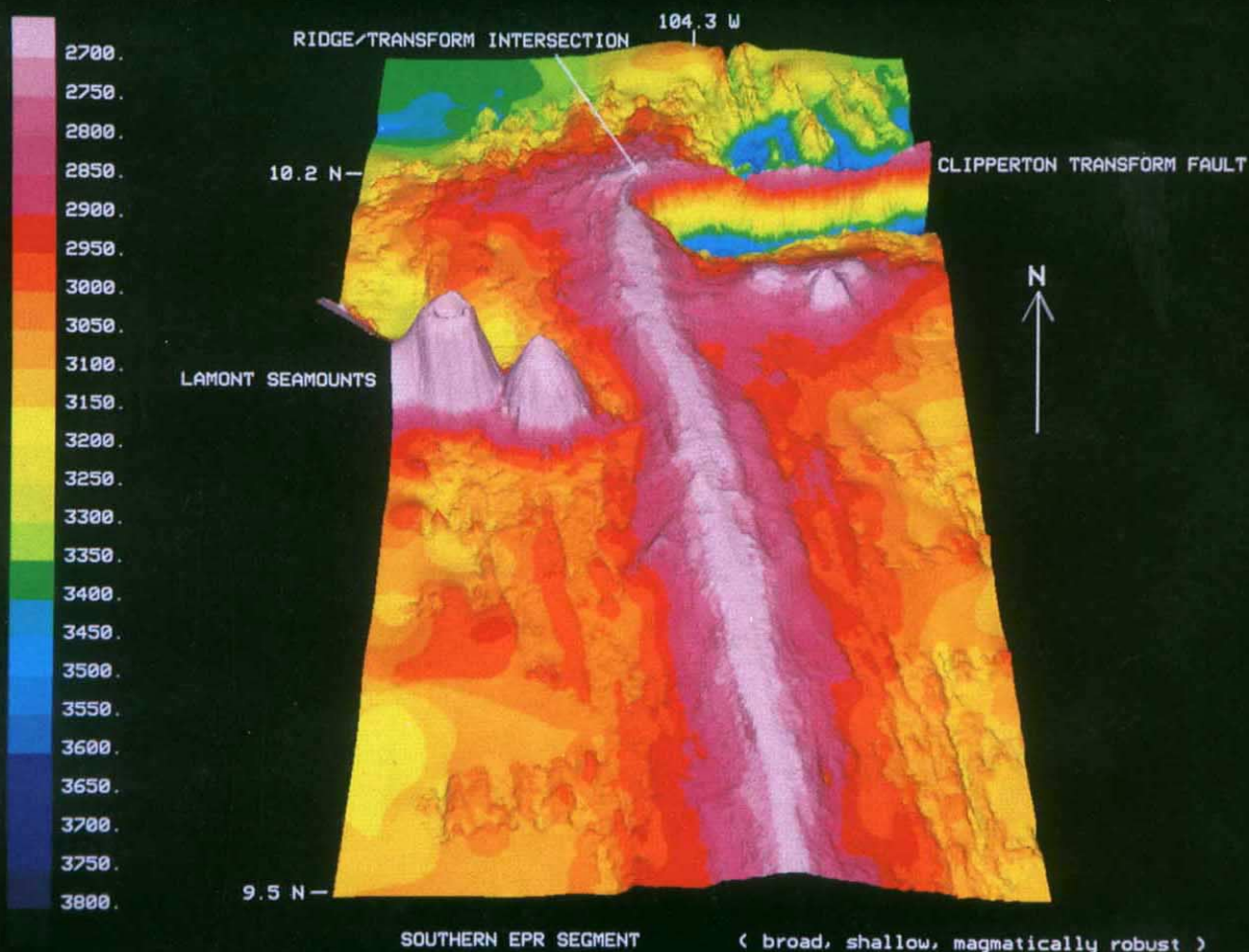


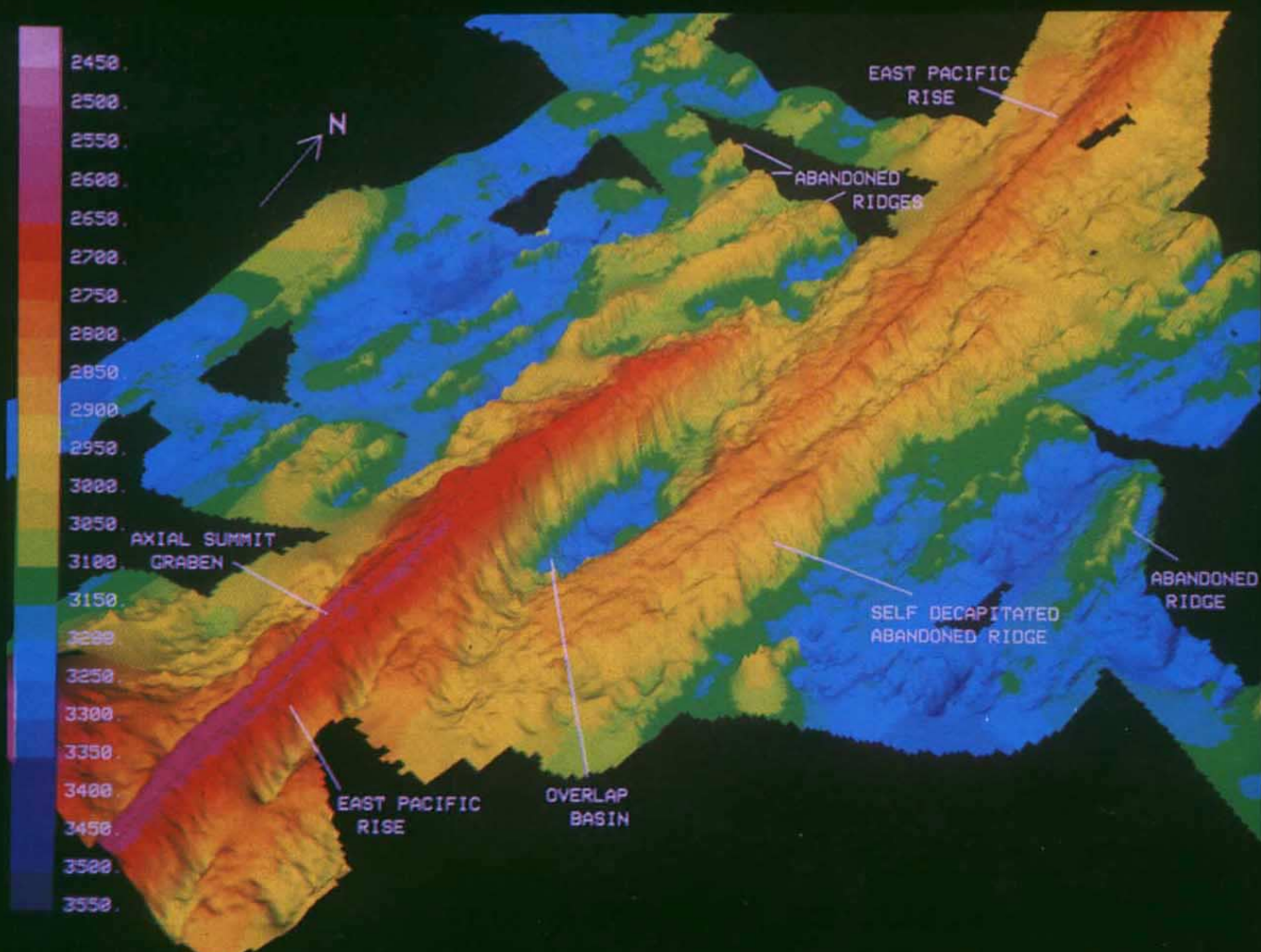
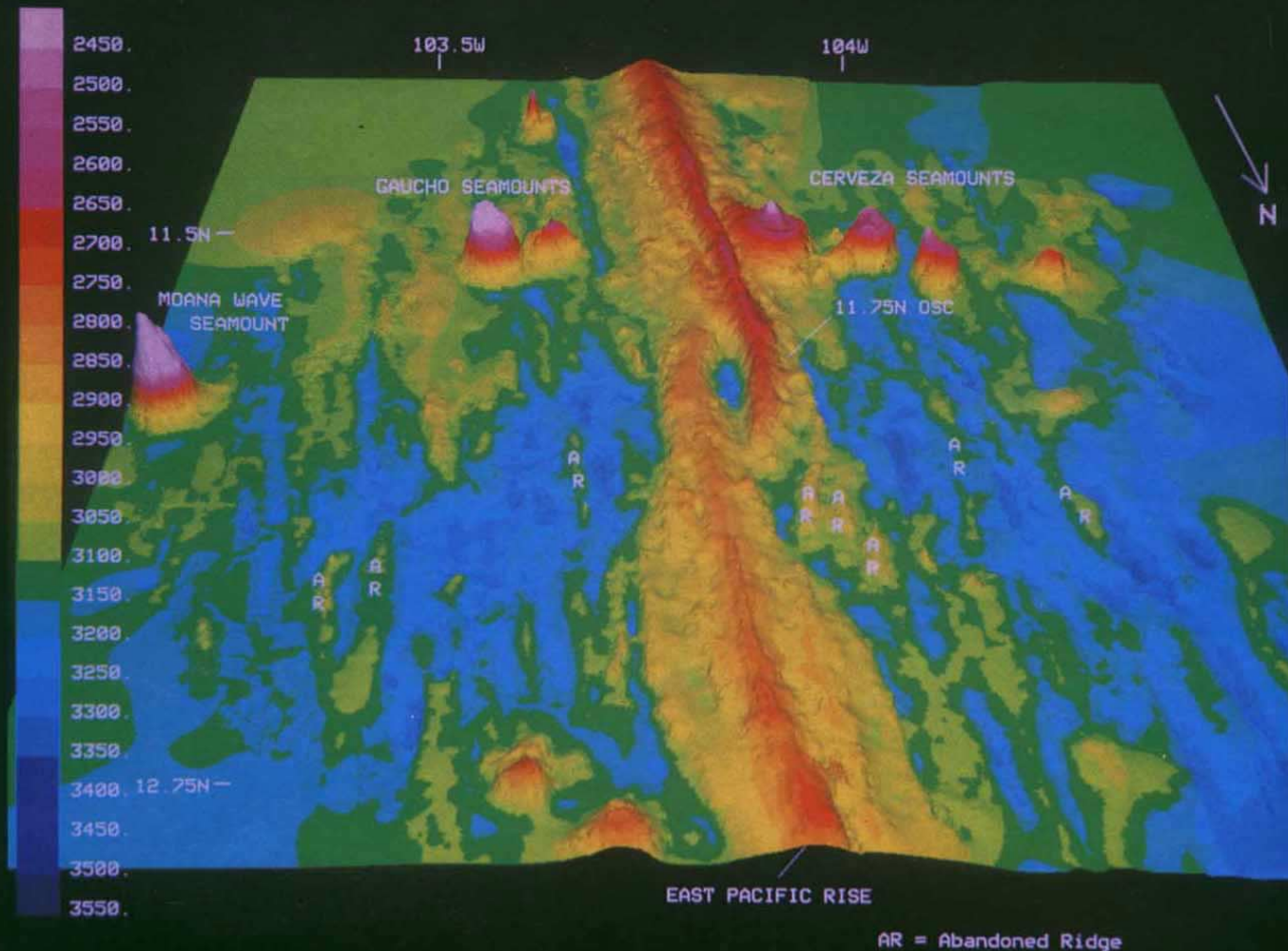
Fig. 2 Shaded-relief images of the Clipperton transform fault (a first-order discontinuity) and adjacent segments of the EPR from gridded Sea Beam data^{6,13} (vertical exaggeration $\sim 10\times$; depths in metres). Sea Beam produces 16 measurements of water depth athwartships over a swath ~ 2 km wide in this area. (The width of the swath is two-thirds the water depth^{3,4}.) Coverage is nearly total; areas with very smooth contours are machine-interpolated data gaps. *Above*, the transform fault is a narrow cleft cutting through the rugged relief of the transform valley joining the two ridge-transform intersections ("RTI"), which are offset by 85 km. Area shown is ~ 100 km (N-S) $\times$ 175 km (E-W). *Opposite page (top)*, the ridge segment immediately north of Clipperton is narrow, triangular in cross-section and plunges over a long distance towards the transform. No magma chamber reflector is detected within 70 km of the ridge-transform intersection; this segment is thought to be starved of magma. Area shown is ~ 100 km (N-S) $\times$ 55 km (E-W). *Opposite page (bottom)*, ridge segment immediately south of Clipperton. The cross-sectional shape of this segment is broad, the depth is shallow and the magma chamber is robust all the way to the ridge-transform intersection. In contrast to the northern segment, this one is magmatically robust. Area shown is ~ 100 km (N-S) $\times$ 65 km (E-W). (Plotting software courtesy of R. Tyce, NECOR/Sea Beam facility.)

flanks of the EPR, achieving continuous coverage over an area of 202,000 km² and defining off-axis structures adjacent to over 40 ridge-axis discontinuities (K.C.M., unpublished data). We found that fourth-order ridge-axis discontinuities have no detectable off-axis scars. Fault scarps that flank third-order discontinuities (small OSCs) are often slightly curved in plan view, but this pattern cannot be traced off-axis for more than a few kilometres. The absence of off-axis scars indicates that third- and fourth-order ridge-axis discontinuities must be short-lived ($<10^4$ yr).

In contrast, second-order discontinuities (large-offset OSCs) create unmistakable off-axis scars consisting of cusped ridges and elongate basins, which develop several hundred metres of relief and trend 10–30° oblique to the surrounding normal sea-floor fabric (Fig. 3). These oblique structures are thought to be fossil overlap basins and abandoned ridge tips. The orientation of this discordant terrain does not follow a small circle about

the pole of opening of the two plates, but instead is V-shaped, with the OSC occurring at the apex of the V-pattern (Figs 1 and 3). Evidently, large OSCs migrate along the axis, their velocity ranging from twenty to several hundred millimetres per year, as indicated by the angle subtended by the V-shaped wake (Fig. 1). As not all of the OSCs we mapped migrate in the same direction or with the same speed, they evidently do not adhere to any particular reference frame (such as the hotspot frame). Depending on the OSC, traces of disrupted terrain extend into lithosphere of different ages, from 0.5 to 3 Myr, indicating that all OSCs did not start simultaneously in response to a change in the direction of spreading. Furthermore, a continuous-coverage SeaMARC II survey of the Clipperton fracture zone out to a distance of 120 km on either side of the transform indicates that there has not been a large change ($\pm 3^\circ$) in the direction of spreading during the past 2–3 Myr. However, slight perturbations in the direction of spreading may influence the





sense of offset at OSCs. Note that nine out of ten OSCs on the Cocos-Pacific portion of the EPR (5–18° N in Fig. 1) are right-stepping, consistent with a slight anticlockwise change in the direction of spreading³⁹.

Second- and third-order discontinuities (OSCs) occur where magmatic pulses misalign, and two distinct evolutionary paths are possible (Fig. 6). The dominant spreading centre may cut through to and link with the adjacent *en echelon* ridge, chopping off the opposing ridge tip⁵. In addition, either spreading centre may cut inside or outside of itself, repeatedly slicing off its own ridge tip²⁸. What controls which evolutionary path will be taken? When two cracks propagate toward and then past each other, the crack propagation force drops significantly as the ratio of crack overlap to crack offset exceeds ~ 3 (ref. 29). Applying this relation to spreading centres, we have suggested that lengthening of individual spreading segments may stall when the segment tips overlap by a distance approximately three times their offset (Figs 3a, 4 in ref. 28). If linkage between the segments has not yet occurred, then the next magmatic pulse that propagates along the segment may be deflected away from the path of the existing tip of the segment, slicing off its own ridge tip (self-decapitation). Three self-decapitated ridge tips (or portions thereof) can be seen immediately north-west of the 11°45' N OSC (Fig. 3).

The distribution of structures within the discordant zone of a given OSC is generally not symmetric about the ridge axis (see Fig. 6). The pattern of oblique-trending cusped ridges and elongate basins is best developed on one flank of the ridge. For example, a southward-propagating OSC with a right-lateral offset leaves a trail of abandoned ridge tips and fossil overlap basins primarily on the west flank (Fig. 3; Fig. 6, case 3). The abandoned ridges and basins are far less prominent than the terrain of the active OSC because of cooling and thermal contraction, as well as the rapid isostatic equilibration that occurs in the still very thin and weak lithosphere. Occasionally, abandoned overlap basins and flanking cusped ridges are seen on the east flank (at least one is evident at the 11°45' N OSC). At 11°45' N this appears to be caused by 'duelling propagation'^{30,31}, in which the northern and southern spreading centres alternately dominate, surging back and forth, casting off ridge tips and basins on the west flank when going south (for a right-lateral offset), and on the east flank when going north (Fig. 6, case 3).

Fig. 3 (opposite) Images of the 11°45' N overlapping spreading centre (a second-order discontinuity), which accommodates an 8-km right-lateral offset of the EPR (vertical exaggeration $\sim 10\times$). Top, shaded-relief image of SeaMARCII bathymetric data. Area shown is ~ 140 km (N–S) $\times 130$ km (E–W). Coverage is total in the area shown. The overlapping spreading centre comprises two ridge segments which overlap by approximately three times their offset and are separated by a 600-m-deep elongate overlap basin. The OSC produces an off axis discordant zone which, excluding the abandoned ridges, is ~ 200 m deeper than adjacent terrain, and consists of abandoned ridge tips ("AR") and fossil overlap basins. The V-shaped wake of the discordant zone shows that the discontinuity has migrated south at a rate of $70\text{--}80\text{ mm yr}^{-1}$ over the past 1 Myr. Yet the pattern of abandoned ridge tips shows that adjacent ridge segments have lengthened (and shortened) both northwards and southwards at rates of $\sim 200\text{--}250\text{ mm yr}^{-1}$. Note that the Gaucho and Cerveza seamount chains occur near but not at the discontinuity. Bottom, close-up of the 11°45' N OSC from Sea Beam data⁶. Area shown is ~ 80 km (N–S) $\times 65$ km (E–W). More detail is revealed because Sea Beam has a higher resolution than SeaMARC II³. An axial summit graben is prominent in the foreground on the southern segment and in the background on the northern segment. A soon-to-be abandoned, self-decapitated ridge occurs in the foreground on the eastern flank of the northern segment; its strike relative to the active ridge indicates a southern propagation rate of 250 mm yr^{-1} . Several abandoned ridge tips occur on the western flank of the southern segment, indicating a 200 mm yr^{-1} shortening of this segment.

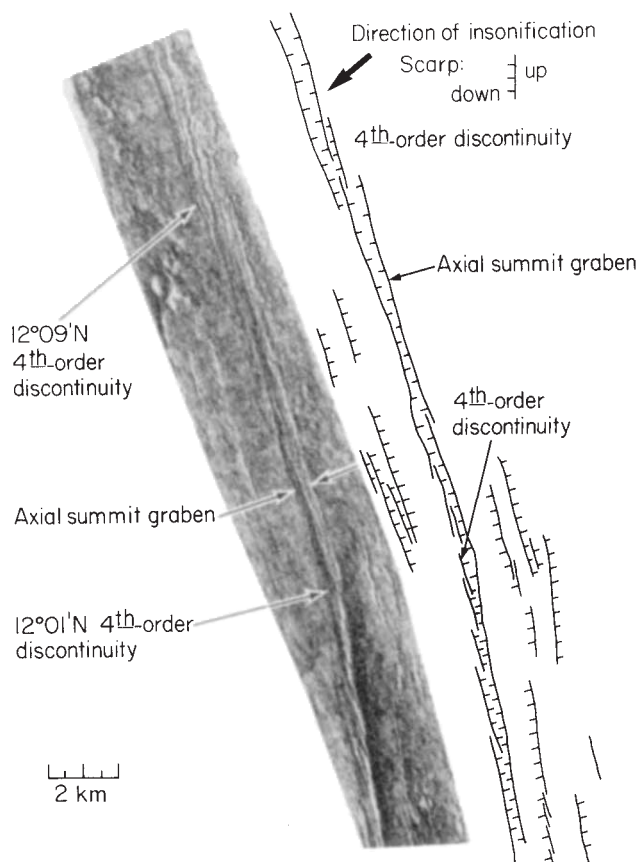


Fig. 5 SeaMARC II side-scan sonar image (left) and interpretation (right) of two fourth-order discontinuities near 12°09' N (a 500-m left-lateral offset of the axial summit graben) and 12°01' N (a 4° change in the local strike of the axis). Dark areas indicate reflected acoustic energy; light areas are shadows. Although the structural signal of fourth-order discontinuities is weak (they are not detectable in the Sea Beam or SeaMARC II bathymetric charts), their geochemical signal is significant^{14,37}.

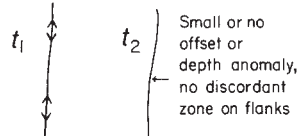
At the 11°45' N OSC, the most recently abandoned ridges indicate a rapid event of southward propagation at a rate of $\sim 200\text{--}250\text{ mm yr}^{-1}$ over the past 0.2–0.3 Myr, and a slower average rate of southern migration of $70\text{--}80\text{ mm yr}^{-1}$ over the past 1 Myr, owing to at least one event of northward propagation (Fig. 3).

Concluding remarks

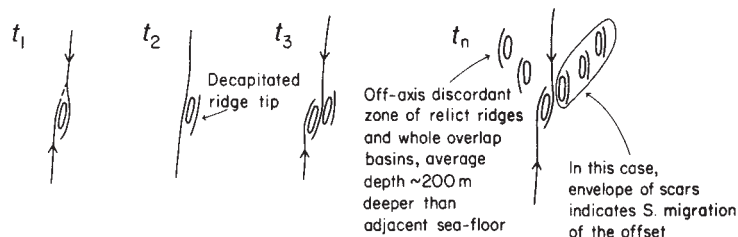
Given that second-order discontinuities have offsets of only 3–10 km, it is surprising how wide a swath of lithosphere they disrupt. The wake of disturbed sea floor at the 11°45' N OSC is 30–50 km wide (Fig. 3). The magnetic field, which is diagnostic of variations in the physical properties of the crust, is disrupted over a region 50–80 km wide centred about the same V-shaped wake as the bathymetric discordant zone, and Vine–Matthews magnetic lineations are not identifiable. In contrast, a major transform fault such as the Clipperton disrupts the crustal fabric and magnetic anomaly pattern over a zone only 20 km wide. Approximately 20% of the sea floor is affected by the wakes of second-order discontinuities. How can such small discontinuities have such a profound influence on crustal structure? They occur away from sources of partial melt, where the axial magma chamber is smallest (averaged over time), and hence thinner, and less long-lived. This will create an oceanic crust that is thinner and more highly fractionated geochemically. In addition, the balance between magma supply and extensional tectonism will change, so that crust created near discontinuities will experience increased stretching. The discordant zone will be wide because of the large spreading-centre overlap (at OSCs

Case 1, Meeting

Magmatic pulses meet head-on, or are aligned but stop short of meeting

**Case 2, Linking and decapitation**

Magma pulses misalign but eventually link

**Case 3, Self-decapitation**

Magma pulses misalign and do not link; ridge tips self-decapitate

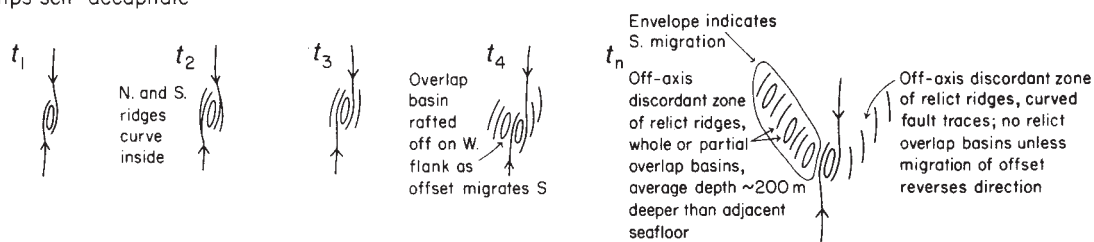


Fig. 6 Summary of three general cases for evolution of ridge-axis discontinuities (modified from ref. 28). Arrows along ridge axis indicate direction of propagation of magmatic pulses and associated cracking fronts. North is towards top of figure; $t_1, t_2, \dots, t_n$ indicate a time sequence. Case 1 applies primarily to fourth-order discontinuities. Cases 2 and 3 apply to second- and third-order discontinuities; both cases 2 and 3 may occur at a given discontinuity at different times. Case 3 may also apply to first-order propagating rifts. For case 3, if magmatic pulses are very frequent (10^2 – 10^3 yr) during an episode of enhanced magmatic activity, then distinct ridge tips may not be decapitated; instead only a sliver of the ridge will be sliced off. Case 3 has been the dominant mechanism at the $11^\circ 45'$ N OSC, including at least one alternation in the direction of migration (see Fig. 3).

in particular) and because of changes in the rate and direction of migration of the discontinuity. In this model, disturbances in crustal structure increase with the size of the discontinuity, although variations in geochemical composition may be pronounced at all discontinuities, regardless of their size¹⁴.

Another important question concerns the factors that control the migration direction of discontinuities and the longevity of a given ridge segment. For five out of six second-order discontinuities where large areas of the flanks have been carefully surveyed, the discontinuity is found to be migrating 'downhill', away from the segment that is both shallower and whose tip has the steeper topographic gradient near the OSC. It has been predicted that the topographic gradient should be the controlling factor³². The one exception is the $11^\circ 45'$ N OSC (Figs 1 and 3), which is migrating south even though the higher topography and steeper topographic gradient lie to the south. In this case, two other factors may play a role in determining the migration direction. The tensile stress intensity at a crack tip is directly proportional to the square root of the length of the crack³³. At $11^\circ 45'$ N, the spreading segment to the north is longer than the segment to the south, at the scale of third-order segmentation (Fig. 1). Thus, there may be a trade-off between the tendency for longer segments to grow at the expense of shorter ones, and the tendency for new, shorter segments to be born when new magmatic pulses feed the ridge. Another factor, which remains highly speculative, is that some melt anomalies in the upper mantle may be tied to a hotspot reference frame³⁴. Most of the recent data collected weigh against this idea (K.C.M., unpublished data), but it cannot be ruled out as one of several factors controlling migration direction. At $11^\circ 45'$ N, for example, the migration direction is broadly consistent with a hotspot frame. At this early stage in our studies, the local magmatic budget, as manifested by the height and gradient of the axial depth profile, seems to be the dominant factor influencing migration direction. Nevertheless, the role of segment length and the possibility of a long-term influence if melt generation is tied to a hotspot

reference frame cannot be ruled out.

There is probably a close relationship between propagating rifts and discontinuities of order 2–4. Propagating rifts are often the product of large-scale reorganization of the plate boundary pattern, usually in response to a change in the pole of relative rotation for the plates concerned⁸. OSCs are ubiquitous along the plate boundary^{6,7} at fast to intermediate spreading rates, and involve rift propagation and concomitant segment lengthening and shortening, but in most cases do not appear to be caused by a change in spreading direction. Changes in spreading direction influence the sense of offset at OSCs, but do not cause OSCs or determine their locations. However, if a change in spreading direction occurs, OSCs may be the nucleation points for large-scale rift propagation, leading to a rearrangement of the plate boundary pattern⁶. In fact, large-offset OSCs (>3–5 km) may develop from third- and fourth-order discontinuities in response to small, short-term perturbations in the spreading direction. Ultimately all of these discontinuities (except transform faults) have a common origin, linked to the upwelling and segregation of mantle melt beneath the mid-ocean ridge which leads to propagation of magmatic pulses along the ridge. Thus the primary differences between the various types of discontinuity, besides morphology and structure, may be their size, duration, fine-scale structural evolution, frequency of occurrence, and whether they accommodate major changes in the configuration of the divergent plate boundary^{31,32}.

The size of a discontinuity and the length of the segment it terminates seem to be linked to its longevity. First-order discontinuities bounding the long-wavelength undulations of the ridge-axis depth profile last at least several million years, and second-order discontinuities last 0.5–3 Myr, but third- and fourth-order discontinuities are very transient. We suggest that the long-wavelength axial topographic gradient (defined by first- and second-order discontinuities) reflects a sustained, long-wavelength pattern of convective upwelling and melt segregation in the upper mantle. Third- and fourth-order discontinuities may

reflect local perturbations in melt delivery. These may be short-lived phenomena, relative to the convective process that supports the long-wavelength pattern. The variation in size, location and longevity of magmatic segments suggests that oceanic crust may be a patchwork (in plan view) of lozenge-shaped igneous units which are each associated with a distinctive set of physical and chemical characteristics in three dimensions, and whose size is

determined by the scale of the parent magmatic segment.

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ARTICLES

Laser-driven implosion of thermonuclear fuel to 20 to 40 g cm⁻³

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Inertial fusion requires the compression of fusion fuel to densities approaching 1,000 times the density of liquid DT. Compression of DT to densities in the range of 100 to 200 times its liquid density has been achieved using cryogenically cooled solid-fuel-layer DT-filled capsules irradiated directly with high-intensity symmetric short-wavelength laser radiation.

THE compression of deuterium-tritium (DT) to high densities represents a critical test of the feasibility of laser fusion¹. An extremely high degree of drive uniformity and symmetry and an ablative low-entropy implosion of fuel capsules are prerequisites for high-density compression. One approach to inertial fusion currently being investigated involves the conversion of laser energy to X rays to drive the implosion (indirect or *hohlraum* drive)². The alternative approach, direct laser irradiation of capsules, may be more energy-efficient than indirect drive if stringent drive uniformity requirements are achieved³. A critical test of the potential of direct drive to meet these requirements has been carried out in cryogenic target experiments conducted on the OMEGA laser facility at the University of Rochester. These experiments, which use a comprehensive set of diagnostics to characterize the time history of the implosion and the compressed-fuel conditions, have resulted in the first direct measurement of high fusion-fuel areal density produced by laser fusion. Compressed DT fuel densities of ~20-40 g cm⁻³, 100 to 200 times the density of liquid DT (100-200 × LD), were achieved, the highest yet attained in direct-drive inertial fusion experiments.

Two sets of implosion experiments were carried out. The first set, in which the fuel was initially in the gas phase, was conducted

to develop, activate and calibrate diagnostics needed to characterize the acceleration phase of the target implosion and to measure the core conditions at the time of neutron production. These experiments produced fuel densities greater than 50 × LD; the results were used for a detailed comparison of theoretical calculations. Details of this series of experiments have been reported previously⁴. For the series of target implosion experiments described here the fuel was initially in the solid phase. Cryogenic cooling was required, together with substantial improvements to both target irradiation and diagnostic techniques, to achieve and measure greatly increased compressed, fuel densities of ~100-200 × LD.

Previous experiments⁵ have reported compressed-fuel densities of ~100 × LD using nuclear activation techniques that measure the shell areal density ($\rho\Delta R$) of the material surrounding the thermonuclear fuel. The compressed-fuel density was deduced by assuming mass conservation, a one-dimensional model of the compressed core and pressure balance, to relate the shell areal density to that of the fuel. In the experiments reported here the fuel areal density (ρR) is measured directly during the time of neutron production. This measurement is insensitive to the shell areal density and to the temperature of the imploded material and is independent of the amount of shell

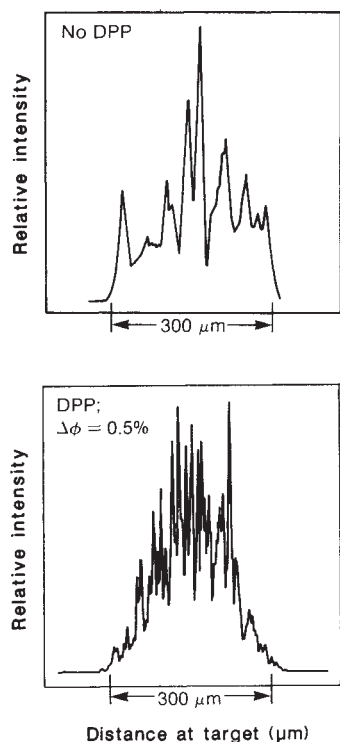


Fig. 1 Line-outs of the target-plane intensity distributions show marked improvement in uniformity (for low-frequency modes) after phase conversion. Numerous hot spots, each about 5% to 10% of the target-plane beam diameter, are replaced by a reproducible intensity envelope that contains small-scale speckle.

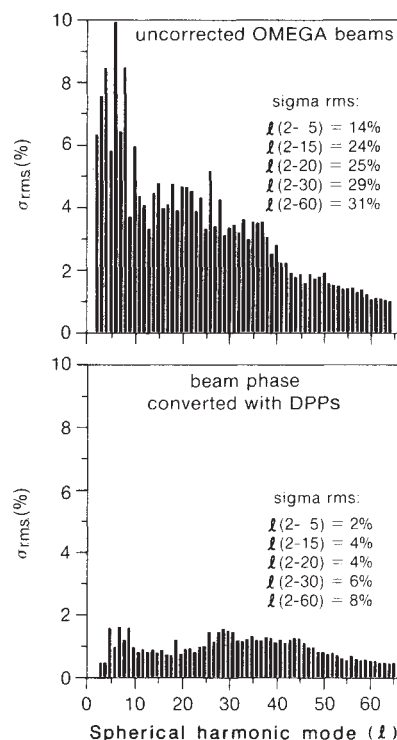


Fig. 2 Overall improvement in the irradiation uniformity on target is assessed by computing a 24-beam superposition of intensities and a spherical harmonic decomposition. The intensity nonuniformity ($\sigma_{r.m.s.}(\%)$) is reduced by a factor of 6 for modes $l=2$ to 20, and by a factor of 4 for modes $l=2$ to 60. Smoothing by 1% of the beam diameter is assumed.

material mixed within the fuel region; it is the first such measurement of highly compressed thermonuclear fuel.

Experimental configuration

The OMEGA laser is a 24-beam Nd:phosphate-glass laser that is frequency-tripled to operate in the ultraviolet (351 nm) using the polarization mismatch scheme⁶. Experiments were performed by irradiating targets with 1,000- to 1,500-J ultraviolet radiation delivered in 600 to 700-ps (FWHM) gaussian-shaped laser pulses. Before these experiments major improvements were made to the OMEGA laser system irradiation-uniformity on target. These improvements were implemented as a result of comprehensive measurements of both beam amplitude and phase, in conjunction with numerical models to simulate sources of target-plane irradiation nonuniformity. Phase defects throughout the amplifier chain, the KDP conversion cells and transport optics were identified as dominant factors controlling the intensity profile in the target plane.

Hot-spot intensity nonuniformities in the OMEGA target plane were found to be caused principally by spatial variations in the near-field phase front of each laser beam. In order to increase the uniformity of target irradiation we implemented, through the use of two-level distributed phase plates (DPPs)⁸, a technique originally suggested by Kato *et al.*⁷ that modifies the phase fronts of the laser beams to produce ~250,000 overlapping 'beamlets' on target. (A DPP is composed of an ordered array of transparent hexagonal elements in which phase retardation is randomly distributed among the elements by using a thin-film layer to introduce optical path differences.) Figure 1 shows cross sections of the target-plane intensity distributions before and after phase conversion with a DPP; the numerous hot spots are replaced by a reproducible and uniform envelope that contains small-scale speckle.

The overall target irradiation uniformity is computed by superposing the intensities from all 24 beams of the OMEGA system. The resulting intensity pattern can be then decomposed into spherical harmonics. Figure 2 shows the computed overall improvement of the target irradiation uniformity through the use of the DPPs. The intensity nonuniformity, $\sigma_{r.m.s.}(\%)$, is improved by a factor of 6 for low-order modes ($2 \leq l \leq 20$) and by a factor of 4 for modes 2 to 60. Smoothing in the plasma by 1% of the beam diameter (~3 μm) is assumed. Beam-to-beam energy balance was ~5%, beam-to-beam timing error was ≤ 3 ps and the individual beam placement on target was accurate to ≤ 10 μm . Based on the above measured quantities and the calculated superposition, the illumination nonuniformity for the entire series of experiments was estimated to be $\leq 12\%$ ($\sigma_{r.m.s.}$).

An integrated cryogenic system, which used the fast-refreeze technique originally developed by KMS Fusion, Inc. (Ann Arbor, Michigan, USA)⁹, was used in the experiments for producing, positioning, protecting and documenting glass shells containing a frozen DT layer. The target-positioning and viewing system allows for three-dimensional positioning of the cryogenically cooled target to within ± 5 μm and measurement of the frozen-fuel-layer thickness and uniformity to within ± 1 μm . The targets were glass shells with a diameter of 200 to 300 μm , wall thickness of 3 to 7 μm . These were filled with DT at 100 atm and which forms a layer ~5 μm thick next to the glass shell when cooled below 19 K. The targets were held at the center of the OMEGA target chamber by spider-silk fibres (<0.5 μm in diameter) drawn across a U-shaped mount. To ensure mechanical stability the target and fibre assembly was coated with a 0.2- μm -thick layer of parylene. The mounted target was cooled to below the freezing temperature of DT in a liquid-He-cooled shroud. The frozen DT layer was prepared further by repeated heating with an Ar-ion laser followed by rapid cooling,

until a layer with a thickness variation of less than 20% was obtained. Approximately 40 ms before target irradiation, the cooling shroud was rapidly retracted, exposing the target to the ambient environment for ~10 ms. (The length of time required for the DT layer to melt was ~30 ms.)

A number of complementary nuclear, X-ray and particle diagnostic systems, including a four-frame high-speed X-ray framing camera and polycarbonate (CR-39) nuclear track detectors, were used to measure simultaneously the target performance⁴.

Experimental results

Measurements of the absorption and the fractional conversion of the absorbed energy into X rays were found to be in good agreement with one-dimensional hydrodynamic code simulations of targets used in these experiments. Typically 60% to 80% of the incident laser energy was absorbed by the target⁴. Measurements of the X-ray conversion efficiency show that approximately 15% to 20% of the absorbed energy was converted to X rays with energies below 9 keV (ref. 4). Time- and space-resolved measurements of the X-ray emission were used to measure the time history of the implosion. The results of these measurements are in general agreement with the one-dimensional code predictions, implying that the actual target drive (the fraction of the absorbed energy converted to kinetic energy of the pusher) is close to that predicted by the simulations. Figure 3 shows the result of a series of X-ray pinhole photographs obtained on a single shot with a four-frame X-ray framing camera¹⁰. Analysis of these data and comparison to the code prediction are shown in Fig. 4. The good agreement between the observed and predicted shell radius as a function of time confirms the accuracy of the theoretical modelling of the fractional incident laser energy converted into shell kinetic energy.

The fuel condition at the time of neutron production was measured with the 'knock-on' diagnostic¹¹. In this technique, the number of deuterium and tritium ions scattered by 14.1-MeV fusion neutrons is detected using a series of polycarbonate (CR-39) track-detector foils¹¹. The number of such ions (knock-ons) is directly proportional to the fuel ρR . Three sets of knock-on detectors were positioned at nearly mutually orthogonal positions about the target, both to increase collecting solid angle and to provide a representative sample of the average knock-on flux. The diameter and length of tracks in the CR-39 foils were

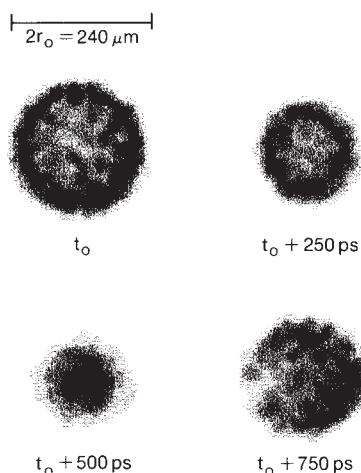


Fig. 3 A set of X-ray images taken on a cryogenic target experiment (number 16054) with the four-frame gated intensifier system coupled to an X-ray pinhole camera. The temporal separation between frames is 250 ps and the frame width is 125 ps. For reference, the first image is taken as zero time (t_0). These images have not been corrected for spatial nonuniformity in the gain of the intensifier.

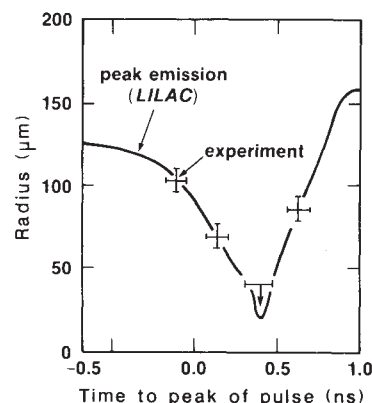


Fig. 4 Measured shell X-ray emission radius versus time determined from the framing camera images in Fig. 3 and one-dimensional (LILAC) code predictions of the same.

used to separate the knock-ons from background protons. This method of background discrimination was tested on low ρR implosions for which there should not be any significant slowing down of the knock-ons in the target; the expected knock-on spectrum is well known from the n-D and n-T elastic-scattering cross sections¹². The measured results were in good agreement with the spectrum predicted theoretically¹¹.

The track selection criteria limit the number of knock-ons detected by a single foil to a discrete energy window that includes only a fraction of the knock-on spectrum. This fraction depends on the distortion of the knock-on spectrum caused by energy loss of the particles as they pass through the target. In general, the knock-on energy loss is a function of ρR , $\rho \Delta R$ and temperature. We used a multi-foil configuration that detects a nearly constant fraction of the knock-on flux, independent of ρR , $\rho \Delta R$ and temperature. Figure 5 shows calculated knock-on spectra for both low ρR_{total} and high ρR_{total} using a five-foil configuration. The deuterons have a longer range than the tritons and therefore can penetrate a higher value of total areal density ($\rho R_{\text{total}} = \rho R + \rho \Delta R$). For the low-density case ($\rho R_{\text{total}} = 20 \text{ mg cm}^{-2}$) the peak of the deuteron spectrum (produced from near head-on collisions between deuterons and neutrons) lies in the window of foil 5, whereas for the high-density case ($\rho R_{\text{total}} = 40 \text{ mg cm}^{-2}$) the deuteron peak has shifted to foil 3. Although the fraction of deuterons detected in any one of foils 2 to 5 differs depending on the degree of slowing down in the target, the sum of knock-ons detected in foils 2 to 5 is a nearly constant fraction, f_D , of the total knock-on number. The fraction is $f_D = 0.085$, to within $\pm 5\%$, for $\rho R_{\text{total}} \leq 50 \text{ mg cm}^{-2}$. This is an upper bound on f_D and is independent of the amount of slowing down. For higher ρR_{total} , f_D will always be less than 0.085 as the deuterons are slowed down out of the windows of the foils. The fuel areal density can be obtained from the number of detected deuteron knock-on tracks by

$$\rho R = 5.4 \times 10^3 \frac{K_D}{Y_N} \left(\frac{4\pi}{\Delta\Omega} \right) \frac{1}{f_D}$$

where K_D is the sum of knock-on tracks detected in foils 2 to 5, $\Delta\Omega$ is the solid angle subtended by the foils and Y_N is the neutron yield. As f_D appears in the denominator of this expression, by using its upper bound we are able to obtain a lower bound for ρR .

The fuel density averaged over the time of thermonuclear burn (neutron-averaged density) can be estimated from the measured ρR by assuming a simple model for the distribution of the fuel in the burn region. One such model (the 'ice-block' model) assumes that the fuel is compressed uniformly into a region of radius R and that neutron production occurs at the

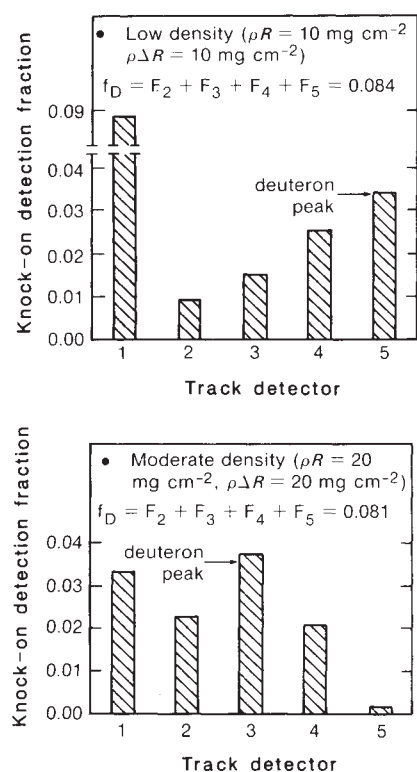


Fig. 5 Relative number of knock-ons in each of the five CR-39 foils for $\rho R_{\text{total}} = 20 \text{ mg cm}^{-2}$ and $\rho R_{\text{total}} = 40 \text{ mg cm}^{-2}$. Each foil is preceded by different amounts of moderating material to bring different parts of the knock-on spectrum into the foil's energy window. For deuterons (D) and tritons (T) the knock-on-energy range (MeV) detected by each foil is: (1) D (6.2–7.6), T (7.3–10.6); (2) D (8.2–9.7), T (9.8–10.6); (3) D (9.4–10.7); (4) D (9.8–11.1); (5) D (11.0–12.6). For $\rho R \geq 5 \text{ mg cm}^{-2}$ all tritons will have slowed down below $\sim 9.8 \text{ MeV}$ so that only deuterons will produce tracks in the energy window for foils 2 to 5.

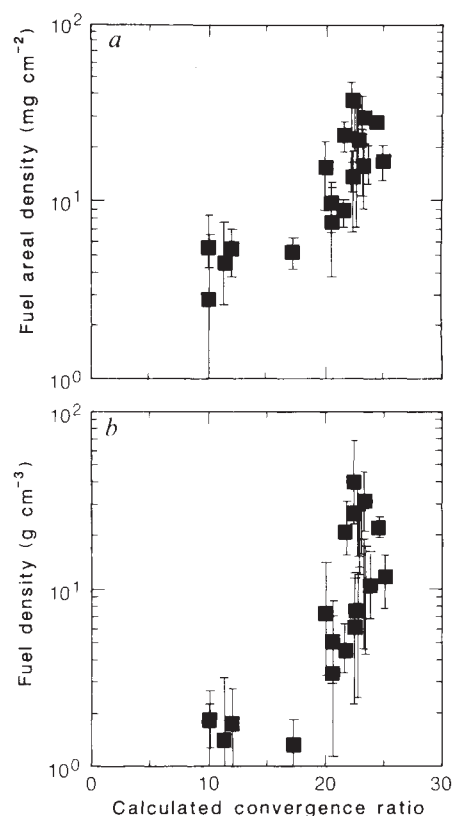


Fig. 6 *a*, Measured fuel areal density plotted as a function of calculated convergence ratio (C_R) for cryogenic target experiments (the convergence ratio is defined as initial fuel-pusher interface radius divided by the minimum fuel-pusher interface radius). *b*, Fuel density inferred from measured fuel areal density plotted as a function of C_R assuming the ice-block model¹³ for the fuel and neutron burn distribution.

centre of this region¹³. Under the assumption that all of the initial mass of fuel, M_i , is compressed into this region, the fuel density is $\rho = (4\pi/3M)^{1/2}(\rho R)^{3/2}$. If any of the assumptions of the model are relaxed, a higher estimate of fuel density would result. For example, if the neutron production were constant across the fuel volume the resulting inferred density would be approximately 50% higher than would be estimated on the basis of the ice-block model. Numerical models of the implosion always suggest a higher fuel density at the edge of the core due to a near pressure-balance at the time of stagnation. This effect will always produce higher average densities for the same ρR than those predicted by the ice-block model.

Measurements of the neutron-averaged fuel areal density were made on a series of cryogenic target experiments. The measured fuel areal densities (obtained from the knock-on diagnostic) and the fuel mass densities inferred using the ice-block model are shown in Fig. 6. The error bars are calculated from the combined relative errors in measurement of the neutron yield and the knock-on flux. The highest values of fuel $\rho R \sim 20$ – 35 mg cm^{-2} . The corresponding fuel densities inferred from these experimental results are ~ 20 – 40 g cm^{-3} .

The observed neutron yields (10^6 – 10^8) and neutron-weighted fuel areal densities (20 – 35 mg cm^{-2}) were below those predicted by one-dimensional simulations. The yields were $\sim 10^{-3}$ of predicted values whereas the fuel areal densities were typically 0.2 to 0.5 of the predicted values. The reasons for these discrepancies are still being investigated, but we suggest that departures from one-dimensional performance are due primarily to residual

imperfections in the level of illumination uniformity presently attained with the OMEGA system.

Summary

A series of direct-illumination, ablatively driven implosion experiments was carried out on the 24-beam 351-nm OMEGA laser system using frozen-fuel DT-filled glass shell targets. Distributed phase plates were used to improve the irradiation uniformity levels on target. Measurements of the energy coupling to the target confirm the high level of collisional absorption and efficient conversion of the absorbed energy to pusher kinetic energy expected with short-wavelength laser illumination. Typical measured absorption fractions were $\sim 60\%$ – 80% of the incident laser energy and are in good agreement with predicted values. Likewise, the measured and predicted shell radius-versus-time were in good agreement with one-dimensional simulations. These results show that gross features of the implosion phase are modelled accurately by one-dimensional simulations.

Using track detectors to measure the elastically scattered fuel ions, fuel areal densities of ~ 20 – 35 mg cm^{-2} were measured directly, implying neutron-averaged fuel densities of ~ 20 – 40 g cm^{-3} (100 – $200 \times \text{LD}$). These are the first direct measurements of the fuel areal density of highly compressed fusion fuel that do not involve any assumptions about temperature or fuel-shell mixing. The inferred fuel densities are the highest attained for any direct-drive laser-fusion experiment.

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Thymic major histocompatibility complex antigens and the $\alpha\beta$ T-cell receptor determine the CD4/CD8 phenotype of T cells

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T-cell receptors and T-cell subsets were analysed in T-cell receptor transgenic mice expressing α and β T-cell receptor genes isolated from a male-specific, H-2D^b-restricted CD4⁺8⁺ T-cell clone. The results indicate that the specific interaction of the T-cell receptor on immature thymocytes with thymic major histocompatibility complex antigens determines the differentiation of CD4⁺8⁺ thymocytes into either CD4⁺8⁻ or CD4⁺8⁺ mature T cells.

THYMUS-derived lymphocytes (T cells) recognize antigen on the surface of antigen-presenting cells in the context of class I or class II major histocompatibility complex (MHC) molecules using the heterodimeric $\alpha\beta$ T-cell receptor (TCR)^{1,2}. CD4 and CD8 molecules, expressed on the surface of T cells, bind to nonpolymorphic portions of class II and class I MHC molecules, respectively, and enhance the binding of the TCR to its ligand^{3,4}. This binding of CD4 and CD8 molecules to MHC antigens may, in addition, contribute to signals leading to T-cell activation.

It is thought that the selection of the antigen-specific T-cell repertoire involves the negative selection (suppression or deletion) of autospecific T cells⁵⁻⁸. Some authors have also proposed that T cells are positively selected by thymic MHC antigens such that T cells, emerging from the thymus, bind foreign antigens predominantly in the context of self-MHC molecules⁹⁻¹². To examine both aspects of T-cell repertoire selection we constructed TCR transgenic mice which expressed, on a large fraction of their T cells, a receptor which binds to H-Y antigen in the context of class I H-2D^b molecules. We used monoclonal antibodies that identify the transgenic receptor expressed in these mice to analyse negative selection in male $\alpha\beta$ transgenic H-2^b mice, which express the H-Y antigen as well as H-2D^b molecules. In addition, the analysis of female $\alpha\beta$ transgenic mice which express different thymic MHC antigens should reveal the possible impact of MHC molecules on the selection of T cells in the absence of nominal (H-Y) antigen.

In a previous report we have described our experiments on the mechanism of self-tolerance: from the comparison of $\alpha\beta$ transgenic male and female H-2^b mice we concluded that auto-

specific T cells were deleted in male mice. It was shown that this deletion involved predominantly immature CD4⁺8⁺ thymocytes, which contain the precursors of mature, single positive, CD4⁺8⁻ and CD4⁺8⁺ T cells^{8,13}.

There is less compelling evidence for the positive selection of T cells by thymic MHC antigens in the absence of nominal (H-Y) antigen: there have been reports of T cells recognizing foreign antigens predominantly in the context of those MHC molecules which they encountered during their maturation in the thymus⁹⁻¹². It was also reported that animals that received large doses of class II MHC-antigen-specific antibodies were devoid of CD4⁺8⁻ T cells¹⁴. This could mean that antibodies can interfere with the positive selection of CD4⁺8⁻ T cells by thymic class II MHC antigens. These experiments do not, however, address the question of whether the $\alpha\beta$ TCR is involved in the selection process. On the basis of these and other experiments¹⁵ one of us proposed that the interaction of the TCR on immature thymocytes with thymic MHC antigens will rescue immature T cells from programmed cell death and determine their further differentiation into mature CD4⁺8⁻ and CD4⁺8⁺ T cells. In the absence of nominal antigen, the interaction of the TCR with class II or class I thymic MHC antigens will direct the differentiation of immature T cells into CD4⁺8⁻ and CD4⁺8⁺ mature T cells, respectively^{16,17}. This model predicts that in $\alpha\beta$ transgenic H-2^b mice the transgenic $\alpha\beta$ TCR should be expressed only on CD4⁺8⁺ and not CD4⁺8⁻ T cells because it was originally expressed on a class I-restricted CD4⁺8⁺ T cell which presumably was selected by class I MHC antigens in the thymus of C57B1/6 mice. Here we describe several observations, made in female $\alpha\beta$ transgenic mice, that are consistent with this model.

Firstly, the proportion of CD4⁺8⁺ thymocytes was elevated in $\alpha\beta$ transgenic H-2^b but not H-2^k or H-2^d mice. Secondly, using monoclonal antibodies specific for the transgenic receptor,

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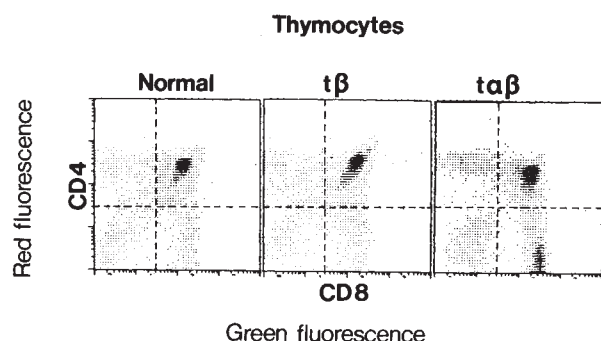


Fig. 1 The proportion of CD4/CD8 thymocyte subsets in female normal, β transgenic and $\alpha\beta$ transgenic mice. Thymocytes were stained by a mixture of phycoerythrin (PE)-conjugated anti-CD4 and fluorescein isothiocyanate (FITC)-conjugated anti-CD8 monoclonal antibodies.

Methods. Transgenic β and $\alpha\beta$ mice of the H-2^b haplotype were produced as previously described^{18,13}. PE-conjugated anti-CD4 (anti-mouse L3T4, ref. 20) and FITC-conjugated anti-CD8 (anti-mouse Lyt-2) were purchased from Becton Dickinson and used at a final dilution of 1 in 50. The staining of thymocytes from female C57B1/6 (normal), transgenic β and $\alpha\beta$ mice was performed as previously described⁸. Two-colour fluorescence was analysed using a FACScan (Becton Dickinson) flow cytometer with a single Argon laser. Ten thousand viable cells were analysed in each sample. Dead cells were excluded from analysis using a combination of low-angle and sideways-light scatter. The results are presented as density plots, generated by analysis of processed data reduced to a 64 × 64 matrix with 16 levels. Where applicable, percentages were calculated using FACScan research software programs. The markers were set against negative controls which involved cells that were incubated in diluent alone and analysed in the same manner as the double-stained cells.

we found that in H-2^b mice only CD4⁺CD8⁺ T cells expressed high levels of both the α and β transgenic TCR chains. In contrast, CD4⁺CD8⁻ T cells expressed high levels of the transgenic β chain only, which was usually paired with endogenous α chains. The new specificity of these receptors allowed the selection of CD4⁺CD8⁻ T cells by thymic class II MHC antigens. Thirdly, in $\alpha\beta$ transgenic H-2^d mice, obtained by back-crossing $\alpha\beta$ transgenic mice to DBA/2 mice, both CD4⁺CD8⁻ and CD4⁺CD8⁺ T cells expressed receptors composed of the transgenic β -chain and endogenous α -chains. The specificity of these receptors allowed the selection of CD4⁺CD8⁻ and CD4⁺CD8⁺ T cells by thymic class II and class I H-2^d MHC antigens, respectively.

T-cell subsets in transgenic mice

The proportions of thymocyte subsets classified by which of the CD4 and CD8 antigens they bear were compared in normal H-2^b and H-2^b mice that expressed either the β or $\alpha\beta$ transgenes. No significant difference was observed between C57L and β transgenic mice. In $\alpha\beta$ transgenic mice, however, the proportion of single positive CD4⁺CD8⁻ T cells was significantly elevated^{8,13} (Fig. 1). These results indicate that the specificity of the transgenic TCR, which was originally expressed by a CD4⁺CD8⁻ T cell, influences the composition of thymocyte subsets. To determine whether the elevated proportion of CD4⁺CD8⁻ thymocytes depended on the interaction of the transgenic receptor with polymorphic domains of thymic MHC antigens, we analysed the composition of T-cell subsets in thymuses of different MHC haplotypes which had been repopulated by haemopoietic stem cells from $\alpha\beta$ transgenic, β transgenic or normal mice. In initial experiments, C57L and B10.BR recipient mice were lethally X-irradiated and repopulated with stem cells from the T-cell-depleted bone marrow of the three different donor mice. When transgenic donor cells were used, most thymocytes

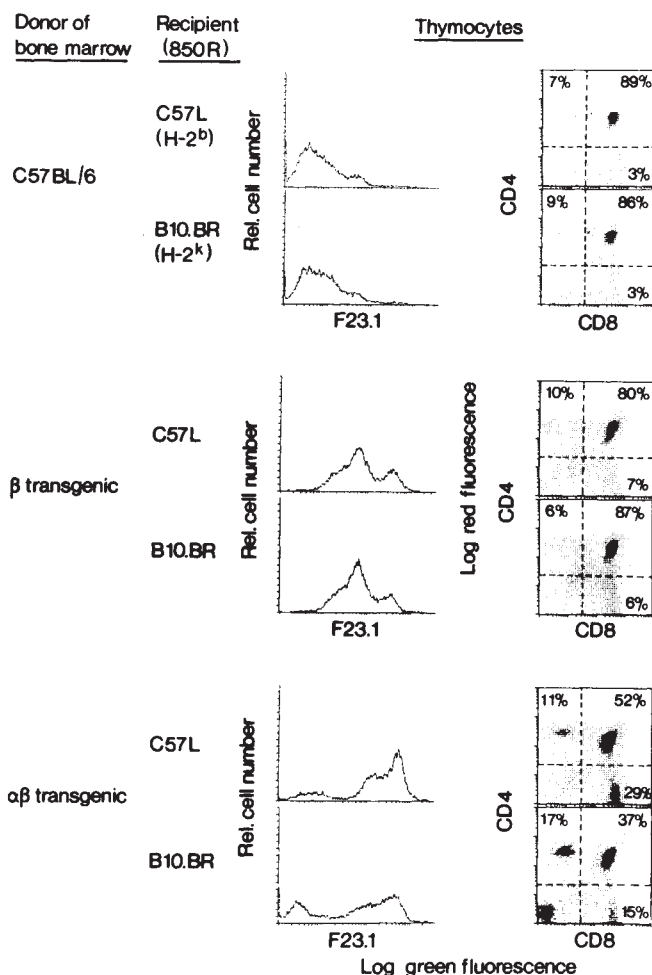


Fig. 2 The expression of transgenic β chain, CD4 and CD8 molecules on thymocytes obtained from various mice.

Methods. Female C57L and B10.BR recipient mice were lethally X-irradiated (850 rads) and reconstituted by intravenous injection of 5×10^6 anti-thy-1 treated bone marrow cells from C57B1/6, β transgenic or $\alpha\beta$ transgenic donors. Both non-transgenic and transgenic donors of marrow cells were of the H-2^b haplotype. After six weeks the thymocytes from the recipients of marrow cells were removed, single-cell suspensions prepared and cells counted and analysed by single and double colour staining. Double staining of thymocytes with anti-CD4 and anti-CD8 antibodies was performed as described in Fig. 1. For single staining with the F23.1 antibody²¹, which detects the expression of the transgenic β -chain, the thymocytes were first incubated with $10 \mu\text{g ml}^{-1}$ of F23.1 monoclonal antibody for 20 mins on ice, washed twice and then incubated with FITC-labelled sheep (Fab^I)₂ fragment anti-mouse immunoglobulin (Silenus Laboratories) at a 1 in 100 dilution for 20 mins on ice. The cells were washed three times and analysed using the FACScan flow cytometer. Five thousand viable cells were analysed in each sample.

expressed the β transgene as detected by staining with the F23.1 monoclonal antibody showing that the repopulation was by donor cells (Fig. 2). The colonization of the H-2^b, but not the H-2^k, thymus by $\alpha\beta$ transgenic cells resulted in an elevated proportion of CD4⁺CD8⁻ cells compared with CD4⁺CD8⁺ cells. This observation was extended in a large series of repopulation experiments involving recipients expressing H-2^b MHC antigens (C57L, C57B1/6, B10.HTG) and recipients lacking H-2^b MHC antigens (B10.BR, B10.D2, DBA/2). We consistently found that only those thymuses that expressed H-2^b MHC antigens and were repopulated by $\alpha\beta$ transgenic stem cells, had a higher proportion of CD4⁺CD8⁻ thymocytes. We also observed that the

thymuses of MHC-mismatched ($H-2^k$ or $H-2^d$), but not partly mismatched ($C57B1/6 \times DBA/2$) F_1 recipients, contained fewer thymocytes (10–20%) than thymuses of $H-2^b$ animals. Furthermore, in completely allogeneic thymuses, $\alpha\beta$ transgenic stem cells yielded only one-third to one-half of the progeny of normal stem cells. Despite differences in absolute cell numbers, which may depend in part on a reaction of MHC-mismatched recipient cells towards donor cells, mice expressing $H-2^b$ antigens in their thymus always had an elevated proportion of $CD4^+8^+$ thymocytes, and they were the only ones to do so.

We adopted an alternative approach to document the influence of thymic MHC and the specificity of the TCR on the development of thymocytes and back-crossed $\alpha\beta$ transgenic animals to $DBA/2$ mice (see below). In this case the thymuses of $\alpha\beta$ transgenic $H-2^d/H-2^d$ homozygous but not $\alpha\beta$ transgenic $H-2^d/H-2^b$ heterozygous mice contained normal numbers of thymocytes as well as a normal ratio of $CD4^+8^-$ to $CD4^+8^+$ thymocytes (3:1 to 10:1). Taken together, the results indicate that the specificity of the TCR, as well as thymic MHC antigens determine the subset composition of thymocytes.

T-cell receptors in transgenic $H-2^b$ mice

If an interaction of the transgenic, heterodimeric TCR with thymic $H-2^b$ MHC antigens was responsible for the elevated proportion of $CD4^+8^+$ thymocytes, most of these cells would presumably express both transgenic TCR chains. In contrast, one would expect that the interaction of TCRs with thymic class II MHC antigens, required for the selection of $CD4^+8^-$ cells, would depend on the expression of endogenous TCR chains generating TCRs with new specificities. We have previously shown that in our transgenic mice the β transgene prevents the rearrangement of endogenous V_β genes¹⁸. Thus, new specificities can result only from the rearrangement and expression of endogenous V_α genes, which was observed in our $\alpha\beta$ transgenic mice¹³. To investigate the expression of α -TCR genes on various T-cell subsets we prepared a monoclonal antibody that detects the transgenic α -chain. For this purpose we immunized BALB/B mice with the B6.2.16 clone from which the α and β transgenes were isolated. A B-cell hybridoma, referred to as T3.70, was obtained by fusing the immune spleen cells with the myeloma cell line AG8.653. This hybridoma produced antibodies which

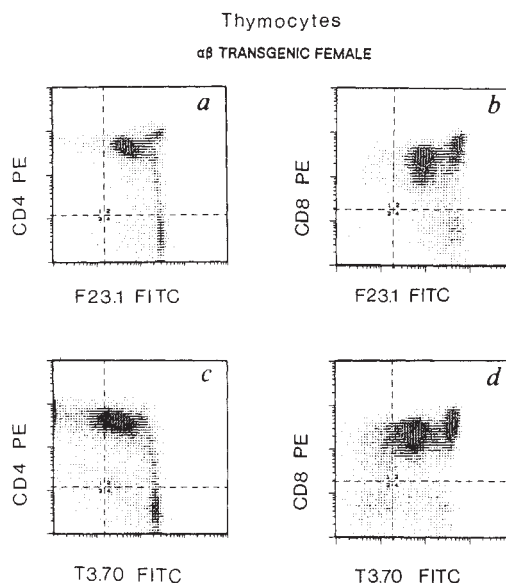


Fig. 4 Staining of thymocytes from female $\alpha\beta$ transgenic $H-2^b$ mice by F23.1, T3.70, CD4 and CD8 antibodies. Thymocytes (yield 1×10^6) were doubly stained for F23.1 and CD4 or CD8 (a and b) and for T3.70 and CD4 or CD8 (c and d).

Methods. Thymocytes were double stained with F23.1 and anti-CD4 or anti-CD8 monoclonal antibodies by incubating the cells first with unconjugated F23.1 followed by FITC-labelled sheep (Fab')₂ fragment anti-mouse immunoglobulin. To saturate free mouse immunoglobulin binding sites for the FITC-labelled antibody the cells were incubated with whole mouse serum (2% v/v) for 20 mins after the FITC step. The cells were then stained directly with PE-labelled anti-L3T4 or with biotin-labelled anti-Lyt-2 followed by streptavidin phycoerythrin (both from Becton Dickinson). A similar procedure was used to stain thymocytes with T3.70 and anti-CD4 or anti-CD8. Non-specific binding of PE to the splenic T cells was minimized by washing the cells four times after incubation with the biotinylated antibody. Two-colour fluorescence was analysed as described in Fig. 1. Proportions of single positive cells expressing high levels of the F23.1 or T3.70 idiotype were: $CD4^+$, F23.1⁺ > 95%; $CD8^+$, F23.1⁺ > 95%; $CD4^+$, T3.70⁺ < 5%; $CD8^+$, T3.70⁺ > 90%.

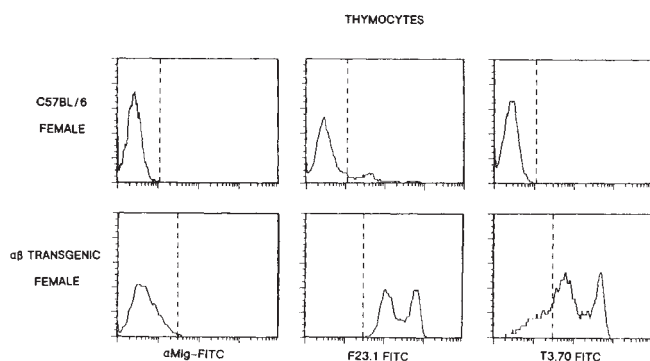


Fig. 3 Staining of thymocytes from female $C57BL/6$ and $\alpha\beta$ transgenic $H-2^b$ mice by F23.1 and T3.70.

Methods. Thymocytes from a female $\alpha\beta$ transgenic mouse were incubated with phosphate-buffered saline, F23.1 or T3.70 followed by incubation with FITC-labelled second antibody as described in Fig. 2. The stained cells were then analysed using the FACScan flow cytometer. The markers were set against thymocytes that were incubated with the FITC-labelled second antibody alone. Five thousand cells from each sample were analysed. In this experiment the percentage of thymocytes stained specifically by the F23.1 and the T3.70 monoclonal antibodies were 99.6% and 76.1%, respectively. The percentage of $C57BL/6$ thymocytes stained by F23.1 and T3.70 were 10.2% and 0.0%, respectively. α MIg is anti-mouse immunoglobulin.

stained the B6.2.16 T-cell clone but not the 93.2.20 T-cell clone (derived from a β transgenic mouse), T cells from normal $C57BL/6$ mice or T cells from α transgenic mice; it also precipitates a disulphide-linked heterodimer with a relative molecular mass of 90,000 (in preparation). Thus the T3.70 antibody seems to be specific for an idiotype determinant that is dependent on the co-expression of both the α and β transgenic TCR chains.

Single staining of thymocytes from female $\alpha\beta$ transgenic $H-2^b$ mice shows that they express low and high levels of the idiotypes recognized by either the F23.1 or T3.70 antibodies (referred to as F23.1 and T3.70 idiotypes). We already know that low receptor levels are found on $CD4^+8^+$ thymocytes. It is also clear from Fig. 3 that some thymocytes do not bear the T3.70 idiotype but are F23.1 positive, and therefore do not express the transgenic α -chain. Because α - and β -chains are present in equimolar concentrations on T cells this is consistent with our previous observation that some endogenous α genes are being expressed by T cells from $\alpha\beta$ transgenic $H-2^b$ mice. In further experiments the differential expression of the transgenic α -chain on thymocyte subsets was analysed by double staining with one of the F23.1 or T3.70 antibodies and one of the CD4 or CD8 antibodies. From the data in Fig. 4 it is apparent the majority of $CD4^+8^+$ cells express low levels of both the T3.70 and F23.1 idiotype and therefore low levels of both α and β transgenic TCR chains (Fig. 4a–d). $CD4^+8^-$ thymocytes express high levels of the

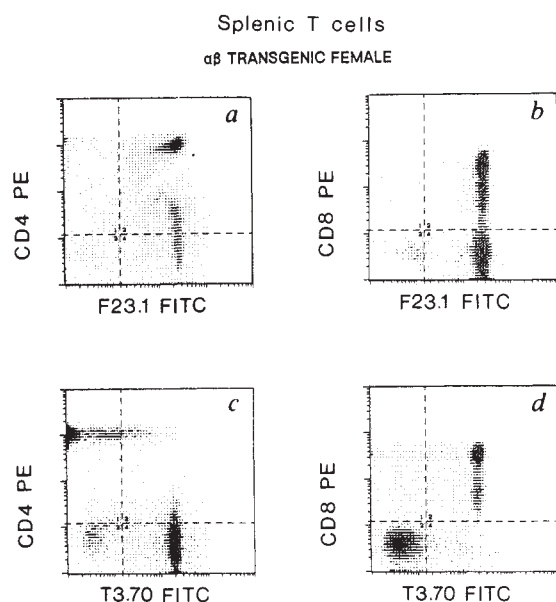


Fig. 5 Staining of peripheral, nylon-wool-purified splenic T cells from female $\alpha\beta$ transgenic H-2^b mice by F23.1, T3.70, CD4 and CD8. Nylon wool-nonadherent spleen cells from a female $\alpha\beta$ transgenic mouse were double stained with F23.1 and CD4 or CD8 (a and b) and with T3.70 and CD4 or CD8 (c and d).

Methods. Spleen cells from a female $\alpha\beta$ transgenic mouse were enriched for T cells by passing them over a nylon wool column as described²². The nylon wool nonadherent cells were 88.3% Thy-1⁺ and 9.4% immunoglobulin-positive by single colour FACScan analyses. They were stained and analysed as described in Fig. 4. Proportions of single positive cells expressing high levels of the F23.1 or T3.70 idiotype were: CD4⁺, F23.1⁺ > 95%; CD8⁺, F23.1⁺ > 95%; CD4⁺, T3.70⁺ < 1%; CD8⁺, T3.70⁺ > 90%.

transgenic β - but not α -chain (Fig. 4a and c). In contrast, CD4⁺8⁺ cells express high levels of both transgenic chains (Fig. 4c and d). These results indicate that the selection of CD4⁺8⁺ but not CD4⁺8⁻ cells from immature precursors requires the expression of endogenous α -chains.

The same conclusion is reached from the analysis of splenic T cells from female $\alpha\beta$ transgenic H-2^b mice (Fig. 5): again CD4⁺8⁻ T cells express high levels of the transgenic β -chain but low levels of the transgenic α -chains (Fig. 5a and c) whereas the vast majority of CD4⁺8⁺ cells clearly express high levels of both transgenic α - and β -chains (Fig. 5b and d).

T-cell receptors in transgenic H-2^d mice

Transgenic H-2^d mice were obtained by crossing $\alpha\beta$ transgenic mice with DBA/2 mice and selecting offspring that expressed α and β transgenes and were homozygous at the MHC. As the B6.2.16 clone was obtained from H-2^b mice, we expected that the transgenic TCR will not be selected in H-2^d mice because the B6.2.16 clone is not restricted by H-2^d MHC molecules. Therefore, in H-2^d mice the selection of both the CD4⁺8⁻ as well as the CD4⁺8⁺ subset should depend on the expression of endogenous α genes. The results in Figs 6 and 7 confirm this: we observe that thymocytes from five independent $\alpha\beta$ transgenic H-2^d mice (but not H-2^d × H-2^b heterozygous mice) contain more CD4⁺8⁻ than CD4⁺8⁺ single positive thymocytes (ratio 4:1, Fig. 6a and b) compared to thymocytes from $\alpha\beta$ transgenic H-2^b mice (Figs 1 and 2). Most of the CD4⁺8⁺ thymocytes in H-2^d mice express low levels of both transgenic chains similar to those observed in H-2^b mice suggesting that MHC antigens do not influence the selection of these immature cells. However, the H-2^d mice differ from the H-2^b mice in the levels of α -chain

expression by single positive CD4⁺8⁻ and CD4⁺8⁺ T cells: both subsets lack high levels of the transgenic α -chain (Fig. 6c and d). This is also apparent on lymph node T cells where T cells display wide variation in the level of the T3.70 idiotype, with most cells expressing levels that are much lower (Fig. 7) than those observed on CD4⁺8⁺ T cells from transgenic H-2^b mice (Fig. 5). These data indicate that the selection of all single positive T cells in H-2^d mice requires the expression of endogenous α -chains, and that at least some of the CD4⁺8⁻ T cells in these mice can express relatively high levels of the transgenic α chain (Fig. 7c). This is unlike the situation in H-2^b mice because the expression of high levels of transgenic α - and β -chains in H-2^d mice does not lead to differentiation of immature CD4⁺8⁺ T cells into mature CD4⁺8⁺ T cells.

Discussion

The data reported here provide evidence that the class I MHC-restricted $\alpha\beta$ heterodimeric TCRs and thymic H-2^b MHC antigens are involved in the selection of CD4⁺8⁺ T cells. This selection occurs in the absence of the nominal (H-Y) antigen in $\alpha\beta$ transgenic female mice. The contribution of thymic MHC antigens to this selection process is evident from the fact that an elevated proportion of CD4⁺8⁺ thymocytes is observed in H-2^b, but not in H-2^k or H-2^d, thymuses repopulated by the progeny of $\alpha\beta$ transgenic stem cells. An elevated proportion of CD4⁺8⁺ cells is observed with $\alpha\beta$ transgenic stem cells but not with stem cells from β transgenic or normal C57L mice demonstrating that the $\alpha\beta$ transgenic TCR influences the selection process. CD4⁺8⁺, but not CD4⁺8⁻, cells in H-2^b mice express

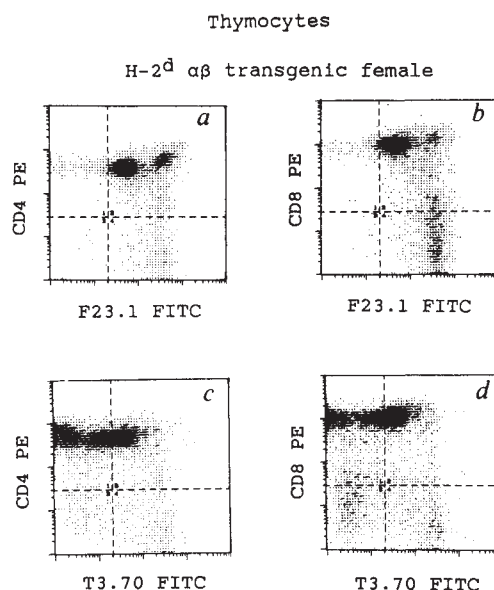


Fig. 6 Staining of thymocytes from female $\alpha\beta$ transgenic H-2^d mice by F23.1, T3.70, CD4 and CD8 antibodies. Thymocytes (yield 7×10^7) were double stained with F23.1 and CD4 or CD8 (a and b) and with T3.70 and CD4 or CD8 (c and d).

Methods. Female $\alpha\beta$ transgenic H-2^d mice were produced by backcrossing the $\alpha\beta$ transgenic founder C57B1/6 × DBA/2. (H-2^{b/d})_{F1} hybrid mouse with DBA/2 (H-2^{d/d}) mice¹³. The H-2 haplotype of the backcrosses was determined by subjecting peripheral blood lymphocytes to killing by specific antisera against K^b or K^d plus complement. Five independent $\alpha\beta$ transgenic H-2^d/H-2^d mice were analysed with similar results as shown here. Thymocytes were double stained with the indicated antibodies and analysed as described in Fig. 4. Proportions of single positive cells expressing high levels of F23.1 or T3.70 idiotype were: CD4⁺, F23.1⁺ > 95%; CD8⁺, F23.1⁺ > 95%; CD4⁺, T3.70⁺ < 5%; CD8⁺, T3.70⁺ < 5%.

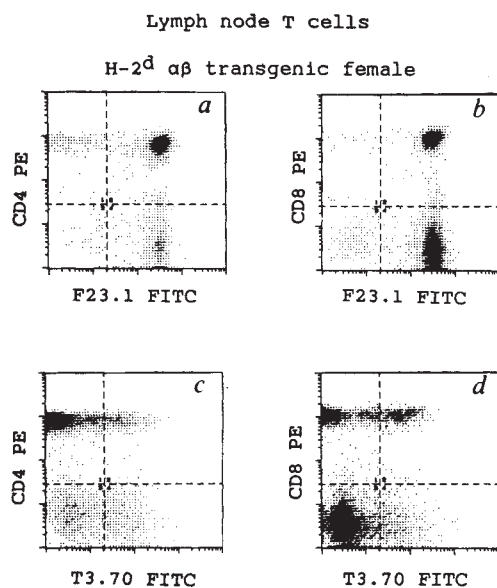


Fig. 7 Staining of lymph node cells (yield 2×10^7) from female $\alpha\beta$ transgenic H-2^d mice by F23.1, T3.70, CD4 and CD8 antibodies. **Methods.** Female $\alpha\beta$ transgenic H-2^{d/d} mice were produced as described in Fig. 6. Lymph node cells were enriched for T cells by passing lymph node cells over a nylon wool column as described²². This preparation of nylon wool non-adherent cells contained 98.9% Thy-1⁺ cells and 0.9% immunoglobulin-positive cells. The immunoglobulin-negative were double stained with F23.1 and CD4 or CD8 (a and b) and with T3.70 and CD4 or CD8 (c and d) as described in Fig. 4. Proportions of single positive cells expressing high levels of the F23.1 or T3.70 idiotype were: CD4⁺, F23.1⁺ > 95%; CD8⁺, T3.70⁺ > 95%; CD4⁺, T3.70⁺ < 10%; CD8⁺, T3.70⁺ < 10%.

high levels of both α and β transgenic TCR chains whereas in $\alpha\beta$ transgenic H-2^d mice both subsets express lower levels of the α transgenic TCR chain confirming the importance of the $\alpha\beta$ TCR. These results are consistent with the hypothesis that the interaction of class I MHC antigens in the thymus with the $\alpha\beta$ heterodimeric T-cell receptor determines the CD4/CD8 phenotype of mature T cells in the absence of nominal antigen.

It is possible that CD4 antigens and the $\alpha\beta$ transgenic receptor are incompatible on the surface of the same cell or that CD4 molecules change the idotype recognized by the T3.70 antibody. This does not seem likely as the majority of CD4⁺ T cells express similar levels of the determinants recognized by the F23.1 and T3.70 antibodies. In addition, this reasoning does not explain the fact that a few CD4⁺ T cells in $\alpha\beta$ transgenic H-2^d mice express high levels of the T3.70 determinant although, in

the same mice, most CD4⁺ T cells express low levels of idiotypes recognized by the T3.70 antibody. It is also possible that the expression of the T3.70 idotype requires CD8 molecules on the cell surface, but this is not consistent with our observation that CD8⁺ T cells and hybridomas express the T3.70 idotype (unpublished results) and, in general, class I-restricted T cells can express a class I-MHC-antigen restricted $\alpha\beta$ heterodimeric receptor in the absence of CD8 molecules¹⁵. It is possible that some interaction of the $\alpha\beta$ heterodimer with the CD8 molecule on immature CD4⁺ T cells is essential for the generation of CD4⁺ thymocytes although this would not explain the elevated proportion of CD4⁺ T cells, which express both α and β transgenic TCR chains, in H-2^b but not H-2^k or H-2^d thymuses. We therefore propose that thymic MHC antigens play an important role in the interaction of the $\alpha\beta$ heterodimer with the CD8 molecule, possibly by cross-linking the two molecules¹⁶, which may lead to the generation of CD4⁺ T cells^{16,17}.

To investigate this further, we will test whether the MHC antigens needed for obtaining a high proportion of CD4⁺ T cells are in fact the restricting class I H-2D^b MHC antigens. It will also be important to determine whether these antigens select CD4⁺ T cells expressing high levels of both α and β transgenic TCR chains. At present, we cannot rule out the possibility that some suppression mechanism interferes with the development of CD4⁺ T cells which express high levels of α and β transgenic TCR chains. We hope to investigate this possibility in $\alpha\beta$ transgenic mice which have been back-crossed to mice with severe combined immune deficiency; such mice should only express the α and β transgenes as these mice are defective in the rearrangement of endogenous TCR genes¹⁹ and are therefore expected to be devoid of any endogenous effector T-cell population including suppressor cells.

The experiments reported here also support our earlier conclusion⁸ that CD4⁺ T cells contain the precursors of single positive cells. CD4⁺ T cells lack high levels of the transgenic α -chain, indicating that these T cells are not male-specific, but their numbers were significantly reduced in male $\alpha\beta$ transgenic mice⁸. The best explanation for this observation is that most of their precursors are deleted in male mice. This implies that the precursors of the CD4⁺ T cells initially express the male-specific, transgenic receptor and, later, rearrange endogenous α loci leading to the expression of new receptors selectable by class II MHC antigens. Therefore, both positive and negative selection can occur at the same stage of T-cell development, that is, negative selection by nominal self-antigen need not occur after positive selection by thymic MHC antigens. These conclusions would imply that the signals leading to positive and negative selection are different.

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Evidence for cyclotron absorption from spectral features in gamma-ray bursts seen with Ginga

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Absorption features near 40 keV in the spectra of several gamma-ray burst sources, first reported by Mazets *et al.*, have been widely ascribed to cyclotron absorption, implying that a strongly magnetized neutron star is the cause of the bursts. Most interpretations of the spectra have been hampered by instrumental limitations: the absorption features are usually at the lower end of the energy range covered by the detector, making spectral unfolding unreliable. Here we report new observations by the gamma-ray burst detector on board the Ginga satellite, which has two well-calibrated detectors covering a wide energy range, 1.5 to 375 keV. The spectral features we find are consistent with first and second cyclotron harmonics, which we take as strong evidence for the magnetized neutron star model.

Evidence of spectral features ranging from 30 to 60 keV in gamma-ray bursts observed with KONUS have been reported by Mazets *et al.*¹. Results from HEAO-1 showed another example, albeit with low statistical significance, of a spectral structure near 55 ± 5 keV with a line width of 13 ± 3 keV (refs 2, 3). Because in each case the features appeared at the low-energy end of the region covered by the detector⁴, problems occur with the spectral unfolding and therefore the evidence is not compelling.

After the report of the HEAO-1 result, in 1982, there have been no follow-up observations to confirm the feature in gamma-ray bursts. If it is interpreted as cyclotron emission or absorption, the existence of the feature would provide important evidence for the presence of a strong ($\sim 10^{12}$ gauss) magnetic field in the gamma-ray burst emitting region. Confirmation of this feature is therefore an important goal⁵.

Investigation of gamma-ray burst spectral features was one of the primary objectives of the gamma-ray burst detector (GBD) on board the Ginga satellite, which was launched on 5 February 1987. The GBD consists of a proportional counter (PC) and a scintillation counter (SC), each with effective area of ~ 60 cm², thus covering a wide energy range from 1.5 to 375 keV (ref. 7). The PC covers 1.5–28 keV with 16 channels and the SC covers 14–375 keV with 32 channels. This energy range assignment, together with a careful pre-launch calibration of the energy responses of the two detectors, gave us confidence that spectral features could be identified in the energy range 15–150 keV. Further details of the instrumentation are described elsewhere^{6,7}.

Since the start of observation, more than a dozen gamma-ray burst candidates have been observed. One of these events (GB880205) shows clear spectral features near 20 and 40 keV, and another (GB870303) shows similar features but with less significance. In Fig. 1 we show the counting-rate evolution of the PC and SC for GB880205; time resolution is 0.5 s and 31.25 ms respectively. The time profiles were divided into 3 sections of 5.0 s each (labelled *a*, *b* and *c*). For each section, observed energy spectra (raw counting rates) were obtained and

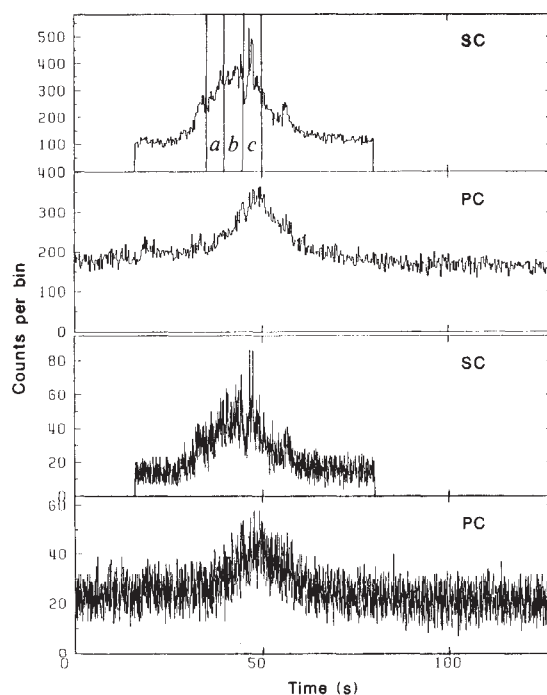


Fig. 1 Counting rate profiles of GB880205 by the SC and the PC (time resolution 0.5 s and 31.25 ms respectively). The PC(SC) covers an energy range of 1.5(14) to 28(375) keV. *a*, *b* and *c* indicate time sections corresponding to the spectra shown in Fig. 2. Time interval is from -32 to $+96$ s of the burst onset time.

corrected for background (Fig. 2). A spectral feature can be clearly seen in the spectrum for section *b*. The probability that this spectrum could be produced by a smooth continuum is $< 10^{-3}$.

The spectral responses of the GBD have been checked by observing the Crab nebula, for which no such spectral feature was seen. The gain of each counter has been monitored continuously using radioactive materials attached to the counters and was found to be very stable⁷. We conclude that the observed spectral features are not due to any instrumental effect.

These features have two possible interpretations: (1) They consist of a broad absorption feature which covers 10–50 keV centred near 30 keV, plus a narrow emission line at 26 keV. In this case the width of the narrow emission line is consistent with the detector response of 20% full width at half maximum (FWHM) at ~ 26 keV. (2) Two absorption lines centred at ~ 20 keV and ~ 40 keV with line widths of ~ 4 keV and ~ 14 keV (FWHM) respectively. Interpretation (2) is reproduced by fitting two gaussian absorption lines with a thermal cyclotron spectrum (compare ref. 8) as the continuum during section *b*. This fitting yields line positions of 19.3 ± 0.7 and 38.6 ± 1.6 keV and line widths of 4.1 ± 2.2 and 14.4 ± 4.6 keV (FWHM) respectively. The difficulty of constructing a realistic continuum for (1) leads us to conclude that (2) is more plausible. At a later date we propose to publish more detailed studies on the statistical significance and the fits of the deconvolved spectrum to theoretical models⁹.

The spectrum of GB870303 is shown in Fig. 3. The probability that this spectrum could be reproduced by any smooth continuum is $\sim 10^{-3}$. Fitting the continuum as a thermal cyclotron spectrum yields two absorption features at 20.4 ± 0.7 and 40.6 ± 2.6 keV, with line widths of 3.5 ± 2.7 and 12.3 ± 6.3 keV (FWHM) respectively.

Thus both spectra require absorption lines near 20 and 40 keV. Furthermore, these features seem to appear only in a limited portion of the burst, as shown in Figs 2 and 3. The existence of a second harmonic feature at twice the energy of the first

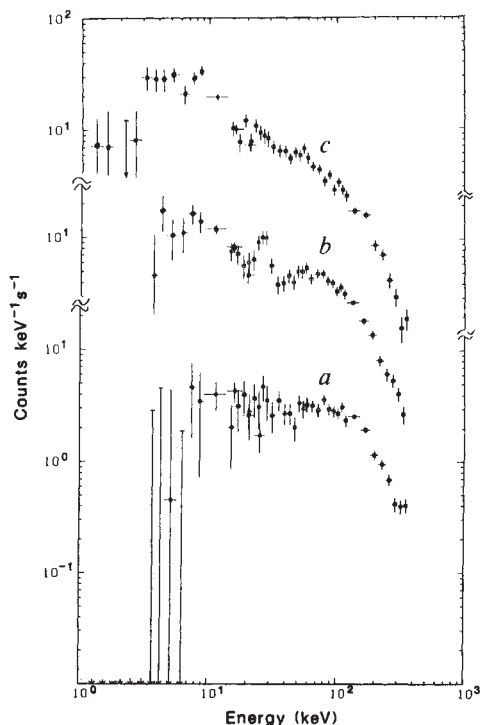


Fig. 2 Raw counting rate spectra of *a*, *b* and *c* (Fig. 1) after background subtraction (not corrected for the counter response). Filled and open circles indicate PC and SC data respectively. The burst spectrum was harder in the early part of the gamma-ray burst and became softer as time progressed. Spectral features centred at ~ 20 and 40 keV can be seen for time section *b*. Deconvolved incident spectrum for this gamma-ray burst will be published later.

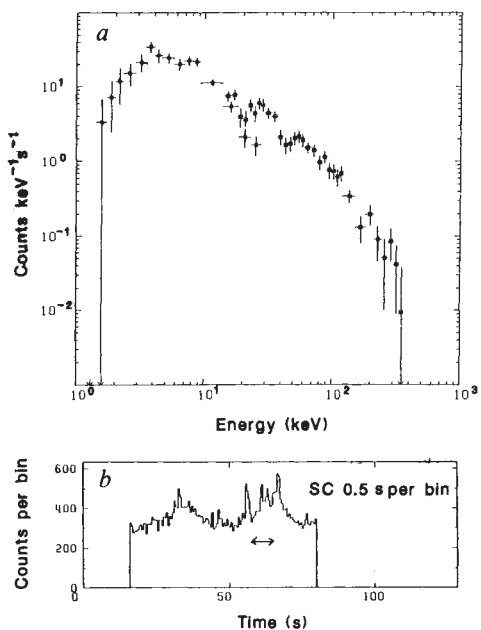


Fig. 3 Raw counting rate spectrum after background subtraction (not corrected for the counter response) (*a*) and raw counting rate history (*b*) of GB870303. The spectrum in (*a*) corresponds to the interval indicated by arrows in (*b*). Spectral features similar to those of GB880205 are seen at ~ 20 and ~ 40 keV.

strongly indicates that cyclotron resonance absorption in a magnetic field is a plausible mechanism for these features. The observed ratios of the line-centre positions are 2.00 ± 0.15 and 1.99 ± 0.33 for GB880205 and GB870303 respectively and are consistent, therefore, with the ratio of 2 expected from the

cyclotron process. Landau levels of a cyclotron harmonic are given by $11.6nH_{12}$ keV (without correcting for relativistic effects and gravitational redshift). For a fundamental harmonic ($n = 1$) of 20 keV a magnetic field of 2×10^{12} gauss is required.

GB870303 and GB880205 have been also detected by PVO, but GB880205 was not detected by a Los Alamos gamma-ray instrument on the DMSP-8 satellite, despite sufficient sensitivity. Using the time-of-flight method, with limits imposed by the fields of view and the Earth's shadow, we can limit the allowed annulus of the source direction. For GB870303 this is a cone of 34.4° centred at (122.4, 19.15) (equatorial coordinate) with a width of 1.6° , and for GB880205 it is an arc between (202, 36) and (142, 28.5) on a cone of 41.3° centred at (175.8, 2.7) with a width of 0.4° . The position of GB880205 limits the incidence angle into the detector to between 15° and 60° (the limit of the field of view).

In summary, our observation of the gamma-ray bursts GB870303 and GB880205 confirms the existence of spectral features at 20 keV and 40 keV. These are interpreted as the fundamental and the second harmonics of a cyclotron resonance, implying the presence of a strong magnetic field in the emission region. The question remains as to why such a narrow absorption feature appears in the gamma-ray spectrum emitted by a high-temperature plasma. We suggest that it is probably produced by a cold region, separate from the hot source region, within the strong magnetic field⁹.

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Radio images of the expanding ejecta of nova QU Vulpeculae 1984

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Nova QU Vulpeculae 1984, with a peak visual magnitude of 5.7 (ref. 1) was one of the brightest and most unusual novae of recent times. Infrared spectrophotometry^{2,3} showed an extremely strong [Ne II] line at $12.8 \mu\text{m}$ and revealed the formation of silicate dust grains in the ejecta 240 days after outburst. The implied high abundance of neon and oxygen prompted the suggestion that nova QU Vul 1984 belongs to a proposed new class of novae in which the underlying system contains an oxygen–neon–magnesium (ONeMg) white dwarf⁴. Radio observations in May 1986 by the

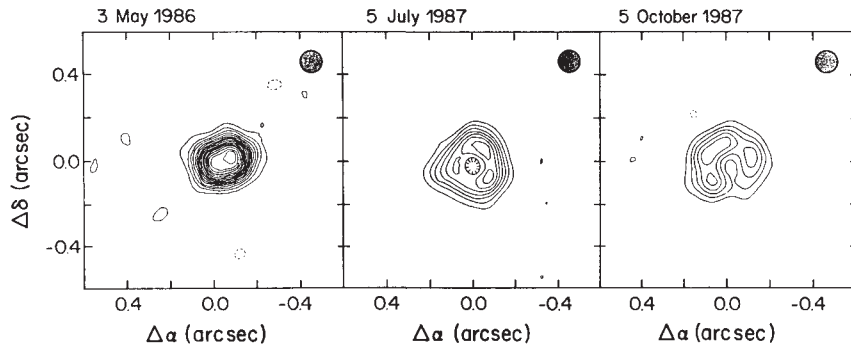


Fig. 1 Radio images of the ejecta of Nova QU Vul 1984 at a wavelength of 2 cm, obtained 497 days (left hand panel), 925 days (centre panel) and 1,017 days (right hand panel) after eruption. The shaded circles at upper right indicate the half-power area of the synthesised beams. The first contour level and the contour interval are the same in all images and equal to 0.6 mJy/beam.

Very Large Array (VLA)⁵ provided the first image of the ejecta, 497 days after outburst. We present here new high-resolution radio images, showing that the ejecta have evolved from a bipolar shape to a spherical configuration with a central hole. The brightness temperature profiles of the new images are well fitted by models with a thick expanding shell of ionized gas with a $1/r^2$ density distribution, and the fitting yields values for the total mass of emitting gas and the expansion rate of the inner and outer edges of the shell.

Following the initial radio detection of nova QU Vul 1984 (hereafter QU Vul) about 200 days after optical maximum⁶, the radio flux density has been monitored regularly. Unlike previous radio novae, QU Vul has undergone two distinct outbursts: an early outburst of high brightness temperature that vanished ~ 300 days after the nova eruption, followed by more slowly varying emission that reached peak flux density several hundred days later⁵. High spatial resolution images of the nova ejecta were obtained on three occasions (3 May 1986, 5 July 1987 and 5 October 1987) during the evolution of the second radio outburst. The maps at wavelength $\lambda = 2$ cm on these dates are shown in Fig. 1. In the first image, on day 497, the ejecta had a distinctly bipolar morphology, elongated along position angle 110° with a major axis of 0.23 arcsec and minor axes of 0.13 arcsec (ref. 5). The second observation, 428 days later, shows a remarkably different morphology. The ejecta now appear to be more spherically symmetric, with a diameter of about 0.3 arcsec. The image also shows a central intensity depression.

The morphology of the emission in the two later images indicates a shell of ionized gas with a large central cavity. For two previous novae, FH Ser 1970 and V1500 Cyg 1975, the time dependence of the radio continuum spectrum can be accounted for by a model of a thick expanding shell of ionized gas with a strong density gradient and velocity dispersion⁷⁻⁹. The velocity dispersion causes the thickness of the shell to increase with time such that the inner and outer radii are $r_1 = r_1(0) + v_1 t$ and $r_2 = r_2(0) + v_2 t$. This has been referred to as the Hubble flow model for novae ejecta. For $r_1 < r < r_2$ the gas density distribution follows a power law $\rho \propto r^{-n}$ where $2 \leq n \leq 3$. We have attempted to fit a Hubble flow model to the brightness distribution of QU Vul at $\lambda = 2$ cm on days 925 and 1,017. Assuming spherical symmetry, the brightness temperature of the radio emission is a function only of the angular radius $\theta = r/D$, where D is the distance to the source, and is given by

$$T_B(\theta) = T_e(1 - e^{-\tau(\theta)}) \quad (1)$$

Here T_e is the electron temperature of the ionized gas, which is assumed to be constant throughout the shell. Taking $n=2$ the optical depth may be expressed as

$$\tau(\theta) = 6.5 \times 10^{11} \frac{\nu^{-2.1} T_e^{-1.35} M_T^2}{\mu^2 D^5 (\theta_2 - \theta_1)^2} \int_a^{\sqrt{\theta_2^2 - \theta^2}} \frac{dx}{(\theta^2 + x^2)^2} \quad (2)$$

where ν is the radiation frequency in GHz, M_T is the total mass of the shell in $M_\odot$, μ is the mean molecular weight of the ionized gas, θ_1 and θ_2 are the inner and outer angular radii in

arcsec and the distance D is in kpc. The lower limit to the integral, a , is 0 for $\theta > \theta_1$ and $\sqrt{\theta_1^2 - \theta^2}$ for $\theta < \theta_1$.

The observed brightness temperature distributions, on days 925 and 1,017 averaged azimuthally around the image of the source, are shown by the data points in Fig. 2a and b. The expansion of the ejecta is demonstrated by the fall in the peak brightness temperature from 1,800 K to 1,000 K between the two dates and by the outward motion of the position of the peak. For comparison, the peak brightness temperature on day 497 was 9,900 K. Assuming a distance of 3.6 kpc (ref. 5), we show in Fig. 2 the best fit to the data for a simple, isothermal Hubble flow as a function of θ_1 , θ_2 , M_T and T_e . The dashed line is the brightness temperature of the shell from equation (1), and the solid line is the convolution of the model distribution with the synthesised beam. The agreement between the model and observed distributions is good, except for the inner portion of the shell on day 1,017 where the model overestimates the brightness temperature. Because, in the optically-thin limit, the brightness temperature varies as $T_B \propto T_e^{-0.35}$, this discrepancy could be produced by an inwardly increasing electron temperature, which is not accounted for in our isothermal model. The average electron temperature and total mass of the shell derived from fits to the two epochs agree to within a few percent, and are $T_e = (1.7 \pm 0.1) \times 10^4$ K and $M_T = (3.6 \pm 0.5) \times 10^{-4} M_\odot$ for $\mu = 1.2$. The angular expansion rates of the inner and outer radii of the shell are $\dot{\theta}_1 = 0.051 \pm 0.002$ arcsec yr⁻¹ and $\dot{\theta}_2 = 0.059 \pm 0.002$ arcsec yr⁻¹, corresponding to linear velocities of $v_1 = 880 \pm 40$ km s⁻¹ and $v_2 = 1,010 \pm 40$ km s⁻¹, and a bulk kinetic energy of expansion of a few $\times 10^{45}$ erg.

An additional constraint on the model is the total flux density of the source as a function of wavelength. In Table 1 we compare the model prediction against the observed flux densities at $\lambda = 20, 6$ and 2 cm, measured on days 925 and 1,017, with the VLA. The agreement is excellent. The model also predicts that the shell is optically thin for $\lambda < 2$ cm. Flattening of the radio spectrum above 2 cm is confirmed by observations with the 30-m telescope of the Institut de Radio Astronomie Millimétrique, which indicates a flux density at the time of the VLA observations of ~ 30 mJy at $\lambda = 3$ mm (A. Greve, personal communication).

Although we cannot claim that the Hubble flow model is a unique fit to the data, the geometry and evolution of the ejecta of QU Vul later than 925 days after the eruption are well represented by the model. Unlike FH Ser and V1500 Cyg, however, the evolution of radio emission from QU Vul at earlier

Table 1 Comparison between the model and observed flux densities

Wavelength (cm)	Total flux density (mJy)			
	5 July 1987 Model	5 July 1987 Observed	5 October 1987 Model	5 October 1987 Observed
20	1.7	1.9 $\pm$ 0.2	2.2	2.2 $\pm$ 0.2
6	12.0	11.0 $\pm$ 0.5	11.0	9.0 $\pm$ 1.0
2	22.3	23.8 $\pm$ 1.0	15.9	15.9 $\pm$ 1.0

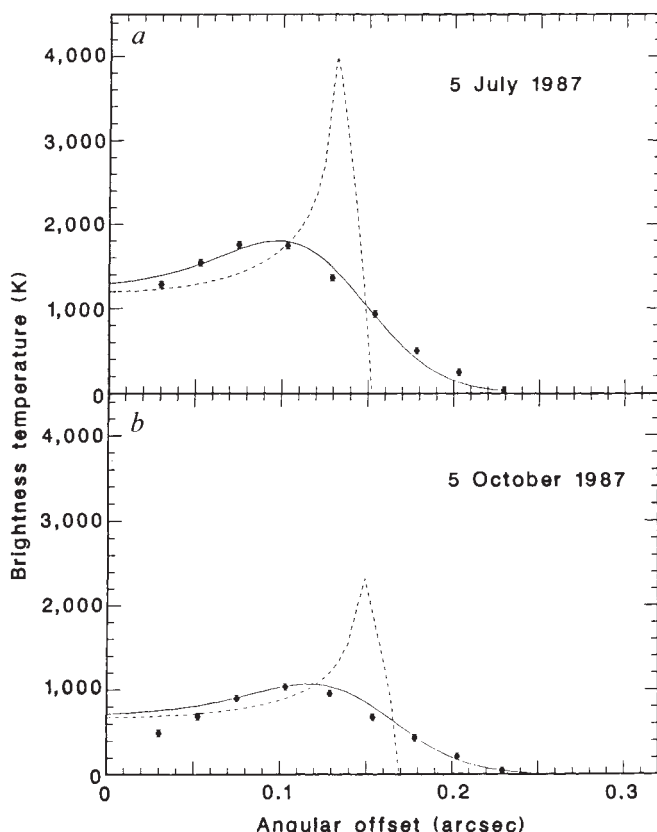


Fig. 2 A comparison of the observed brightness temperature versus angular radius (dots) on days 925, (a), and 1,017, (b), with the model distribution for an isothermal, spherically symmetric, Hubble flow model (solid lines). The brightness temperature distribution of the shell, before convolution with the synthesised beam, is shown by the dashed lines.

epochs is not compatible with the simple evolution of an expanding shell. Backward evolution of the shell solution from day 925 to day 497 yields a much lower flux density than observed. If the radio emission observed up to day 497 and after day 925 arise from the same gas, then some mechanism other than free expansion must have played a role in the evolution of the ejecta. This conclusion is underscored by the fact that the image of the ejecta on day 497 exhibits a bipolar shape. Bipolar ejection from novae, or nova-like objects, has recently been reported by a number of authors¹⁰⁻¹², and Hutchings *et al.*^{13,14} have discussed how the complicated shapes of the optical emission lines from most novae can be produced by a combination of ejection in the form of polar cones and equatorial rings. Non-spherical ejection may thus be a common feature of nova outbursts. QU Vul is remarkable in showing a transformation from an early bipolar source to a spherical source at later times. We now briefly consider possible explanations for this transformation.

The angular expansion rate of the bipolar source, measured up to day 497, was $0.161 \text{ arcsec yr}^{-1}$ along the major axis and $0.095 \text{ arcsec yr}^{-1}$ along the minor axis⁵. At these rates, the angular size of the source on day 925 would be $0.41 \times 0.24 \text{ arcsec}$ —the major axis would be $\sim 35\%$ larger and the minor axis $\sim 20\%$ smaller than the observed diameter of the shell. If the images obtained on the two dates are of the same gas, the material flowing along the major axis on day 497 must have been decelerated and the material flowing along the minor axis accelerated. This seems extremely unlikely. Moreover, the rate of expansion of the bipolar source before day 497 show no evidence for deceleration of the outflow⁵.

A spherical geometry might be produced by strong heating of the ejecta between the two observations. Although the sub-

sequent acceleration of the gas would produce a configuration with larger radius than observed on day 925, if the gas at large radii were very hot and tenuous, it would not be seen in our images. For thermal expansion to dominate the dynamics of the gas, the sound speed must exceed the ejection velocity. A sound velocity greater than $1,000 \text{ km s}^{-1}$ requires a kinetic temperature $T > 5 \times 10^7 \text{ K}$. The unusually strong radio continuum emission shortly after the optical outburst suggested the presence of hot, shocked material⁵, but models of the shock indicated temperatures far below 10^7 K , and the effects of the shock vanished by day 300, long before the time of the first radio image. Evidence for the presence of a significant mass of hot gas at a later stage was reported by Greenhouse *et al.*¹⁵, who detected coronal lines of highly ionized species in the far infrared between days 500 and 800. But, from the ratio of $[\text{Si VII}]$ to $[\text{Si VI}]$ emission they derive a temperature for the coronal zone of only $6 \times 10^5 \text{ K}$. The thermal expansion velocity of this material, $\sim 100 \text{ km s}^{-1}$, is insufficient to affect the shape of the ejecta.

It thus appears difficult to account for the change in geometry by a redistribution of the gas. Another possibility is that the spherical shell was present at earlier times but hidden by overlying material. Combined radio observations from the Very Large Array and the Kitt Peak 12-m telescope show that the emission at $\lambda = 2 \text{ cm}$ on day 497 is optically thick⁵. If the spherical shell expands uniformly, it would on day 497 have been too small to be detectable, being almost entirely hidden within the optically thick surface of the bipolar source. The transformation of the source geometry can thus be explained if the ejecta from QU Vul consisted of two components; an inner, spherically symmetric shell that evolves according to the Hubble flow model, and an outer, more rapidly expanding, bipolar envelope. The fact that the mass of the shell obtained from our brightness temperature analysis is lower than the total mass derived at earlier epochs by Taylor *et al.*⁵ supports this two-component hypothesis.

We must account for the disappearance of the outer, bipolar component by day 925. During the early, optically thick phase of the evolution, the bipolar geometry will dominate the radio emission, but once the bipolar cocoon becomes optically thin, its flux density decays rapidly and emission from the more slowly expanding inner shell will emerge. For an optically thin source expanding at constant velocity, the surface brightness of the radio emission varies as $\Sigma \propto T_e^{-0.35} t^{-5}$, so if the bipolar emission seen on day 497 becomes optically thin shortly thereafter, the peak surface brightness on day 925 would be $\sim 0.5 \text{ mJy per beam}$, which is just below the detection level of the radio image.

Although ejection in the form of an outer bipolar and inner spherical geometry can account for the changing radio characteristics of QU Vul, it is not obvious how such a distribution of material might be produced. The bipolar component may be the remnant of an accretion disk, formerly surrounding the white dwarf of the underlying binary system that was disrupted by the nova explosion. But neither of the two previously studied radio novae show evidence for such a phenomenon, being consistent in both cases with a single, Hubble-flow shell. The number of radio novae is very small, however. Considerable light will be shed on this phenomenon by early and systematic radio observations of future novae.

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Vortex flow in the solar photosphere

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Convective flow fields in the solar atmosphere play a key role in the concentration and dispersal of magnetic flux¹, but because the individual flow elements—the solar granules—are a few arcsec or less in size, studies of their motions have been limited by the distortion and blurring of the Earth's atmosphere ('seeing'). We report here a very high-quality series of granulation images taken at the new Swedish Solar Observatory on La Palma (Canary Islands) which have permitted flow measurements at the sub-arcsec level. These movies show a vortex structure which visibly dominates the motion of the granules in its neighbourhood and persists for the 1.5 h duration of the movie. If such vortices are a common feature of the solar convective zone, they may provide an important mechanism for the heating of stellar chromospheres and coronae by twisting the footprints of magnetic flux tubes.

The vortex we observed had a diameter of ~5,000 km (about three granulation cells), an average circulation of 4,000 km s⁻¹, and a central vorticity of 1,400 s⁻¹. This vorticity is an order of magnitude larger than seen in previous measurements from the Solar Optical Universal Polarimeter (SOU) instrument on Spacelab 2 (ref. 2), which may have been low because of the lower spatial resolution of the smaller telescope. Line-of-sight velocities have been measured spectroscopically using short exposures, requiring high spatial and spectral resolution simultaneously. The vertical component of the photospheric velocity^{3,4} shows a typical root-mean-square (r.m.s.) velocity of 0.2-0.3 km s⁻¹, although correction factors of 3 to 5 are often derived⁵. Doppler measurements of horizontal flows can be made only near the limb, where they are severely compromised by foreshortening effects⁶. The more direct method of measuring horizontal velocities from the displacements of photospheric structures has proved to be particularly difficult with ground-based telescopes^{7,8}. Recent granulation movies from the Solar Optical Universal Polarimeter⁹ (SOU) taken from orbit and therefore free from atmospheric distortions, have allowed measurement of surface flow-fields. Rather than identifying and tracking individual features, as done in the past, the SOU flow-fields were determined using a local correlation-tracking (LCT) technique¹⁰. Although most of the previous usage of LCT

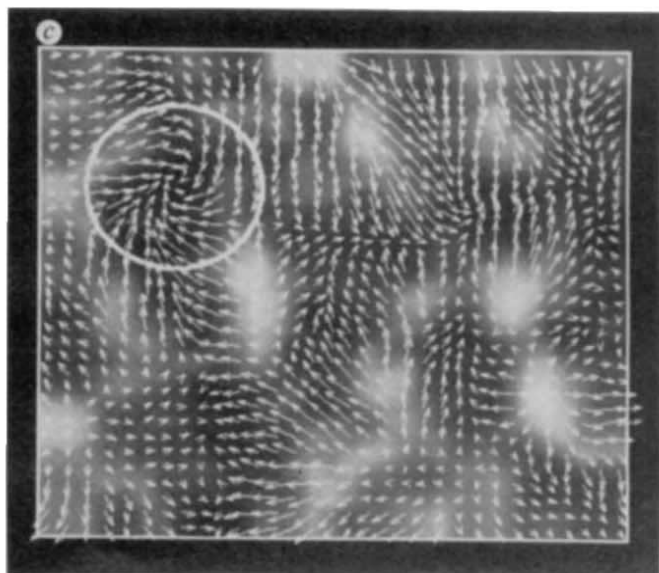
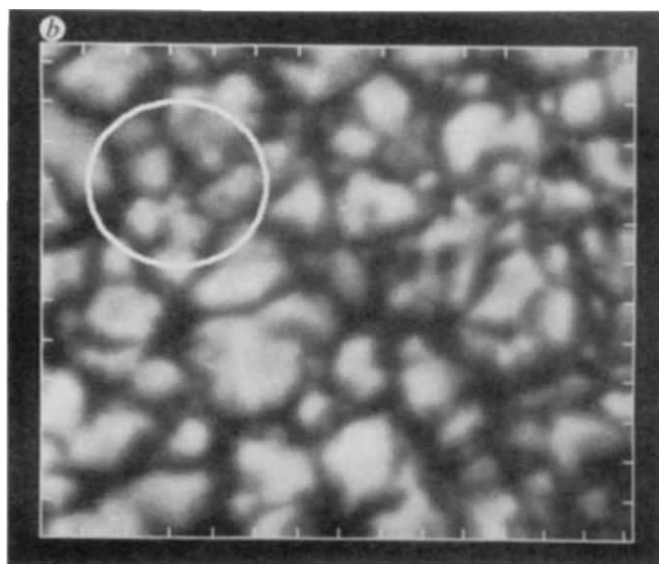
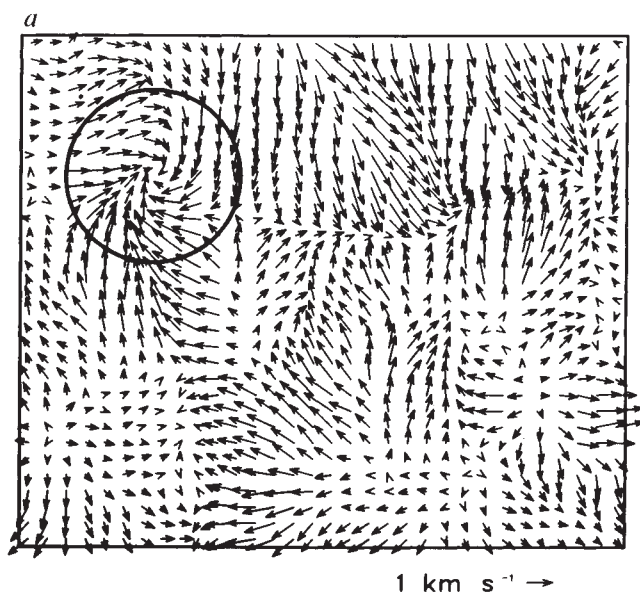


Fig. 1 *a*, Horizontal velocity field derived from granule motions averaged over 79 min. The vortex structure is circled. Arrow lengths are proportional to the velocity; a reference arrow indicating 1 km s⁻¹ is shown below the box. *b*, The solar image for the same field-of-view (14.2 by 12.2 arcsec) taken at the middle of the observing run. The tick marks indicate 1 arcsec (725 km on the Sun). *c*, Same as *a* overlaid on the derived divergence of the flow field. Positive values are bright ('upflow') and negative values are dark ('downflow').

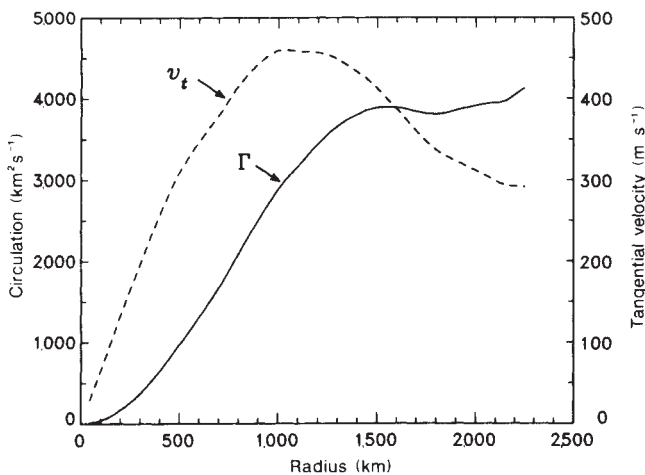


Fig. 2 The circulation Γ (solid line) and the tangential velocity v_t (dashed line) as functions of the radius.

for velocity measurements has been on seeing-free data, analysis of the LCT technique for ground-based images^{11,12} gives us confidence that it can be applied to our high-quality ground-based data.

The new Swedish Solar Telescope on La Palma is located at an altitude of 2,400 m at a site which often has exceptional seeing (0.3 arcsec or better). The 50-cm telescope is of very nearly perfect optical quality. Interferometric measurements using stars¹² show a peak-to-peak wavefront error of $\lambda/15$. Using this instrument, some outstanding time-sequences of granulation images have been obtained recently. The series analysed for this report is among the best and covered the period from 12:41 to 14:00 local time on 16 June 1987. The observed region was near Sun centre and well away from any active regions; however, it is probable that a supergranulation cell-boundary was in the field of view.

The observations were made through a 54-Å filter centred at 4,696 Å. The original images were obtained with a commercial CCD camera with a field of view of 17.8×17.8 arcsec which produced 25 video frames per second. Every 8 s, one of these images was selected for digitizing, using an analogue image sharpness processor interfaced to a MicroVax-based image processing system. A 512×512 pixel array was collected with 8-bit digitization for each of these 8-s intervals. After removal of some bad frames, 388 images were corrected for dark signal and flat-fielded. The image arrays were compressed to 256×256 pixels by selecting only one of the pair of fields per video frame and compressing the line scale by a factor of two. Since only one field of each video image was used for analysis, the effective exposure time was 0.02 s. The resulting images exhibit a r.m.s. contrast between 8.5–10.6%. These images were aligned by cross-correlating sequential images. Because of telescope guiding errors the common field-of-view of the aligned movie sequence was reduced to 14.2×12.2 arcsec.

After alignment the effects of seeing were reduced by destretching the images frame by frame^{11,13}. The seeing distortions were ~ 0.1 arcsec over areas of several arcsec and are very disturbing while observing movies. To remove them each image was divided into 36 sub-images on a ~ 2.3 -arcsec grid. Using a gaussian mask of 2.4 arcsec full width at half maximum, the sub-images were cross-correlated in subsequent pairs for the entire movie. The displacements between subsequent frames, with 12-s average time-spacing, were assumed to be due to seeing effects and the second image of each pair was destretched by cubic interpolation to match the first. This procedure effectively removes seeing and has some effect on large-scale flows.

Local velocities were then determined by measuring the local displacements on a 0.8- and 0.4-arcsec grid on the destretched

and original data, respectively. Displacement velocities were obtained from images separated by ~ 60 s (for each pair the exact times were used). A total of 192 displacement fields were computed for both the original and destretched images. Movies were made of the original, aligned, and destretched images. In addition, movies were created of the displacement field and of the displacement field overlayed on the destretched images. Visual inspection of the movies showed that the inferred velocity fields were qualitatively correct.

To verify the velocity measurements further, a grid of free particles was displaced by the average flow-field. At time zero this grid had a spacing of 0.8 arcsec. The points of the grid were displaced to the time of the second frame according to the velocity at each point. The flow field on the second frame was interpolated to the new locations of the sample points, which were then moved to the time of the third frame according to these velocities. This process was continued for the duration of the flow sequence. A movie of the test particles was made and overlayed on the destretched movie. The test particles verified in detail that the calculated flow-field measured the evolution of the intensity field.

Averaging the velocity vector at each point in the grid for the 79-min observing period yields the horizontal flow-field (Fig. 1a). In Fig. 1 we have used average velocities computed from the original rather than the destretched data, although the two sets of data yield velocities that do not differ significantly. Figure 1b shows the granulation pattern for a single good frame, and Fig. 1c shows the flow field overlayed on its divergence, which is roughly proportional to the vertical component of the flow⁹. The resolution of the flow map is about 0.5 arcsec, 360 km. Of particular interest is the vortex structure contained in the 3,000-km diameter circle in the upper left-hand corner of the image. There are also several strong linear downflow regions and strong sink regions. In addition there is an overall steady flow-pattern with a size of 5–7 arcsec, which is the scale of mesogranulation^{8,14–16}.

Average flow-maps for sequential 20-min segments exhibit essentially the same geometry. Flow speeds in the 79-min average map range from a few hundred m s^{-1} to 1.2 km s^{-1} with an average of 0.67 km s^{-1} . The average speeds of the 20-min average maps range between 0.75 and 0.87 km s^{-1} . The r.m.s. flow speed, corrected for noise, in the individual flow-maps is 1.58 km s^{-1} ; the r.m.s. noise level in the individual maps has been estimated from power spectra to be 0.4 km s^{-1} . These speeds are much larger than reported previously¹⁷, mainly because of the higher resolution of our data and smaller field-of-view used in local correlation tracking. When SOUP data are reanalysed with smaller fields-of-view, the speeds increase to values consistent with our results. Some of the velocity measured by correlation tracking at this resolution might be due to phase and wave effects. We will discuss details of the properties of the 'normal' flow-field in a subsequent paper, but we are convinced that the LCT technique accurately measures the geometry of the motion of the granulation intensity field.

The most novel feature of the movie was the discovery of a vortex flow pattern. This feature covers an area of approximately $7 \times 10^6 \text{ km}^2$, and its circulation Γ of $4,000 \text{ km}^2 \text{s}^{-1}$ remains stable to $\pm 20\%$ over the 79 min of observation. Here the circulation Γ is defined as

$$\Gamma = \oint_C \mathbf{v} \cdot d\mathbf{l}$$

where $\mathbf{v}$ is the velocity and $d\mathbf{l}$ is the line element along the curve C . By Stokes' theorem, Γ also equals the integral of the vertical vorticity within the area bounded by C . Figure 2 shows the circulation around a circle centred on the vortex as a function of radius (solid line), and the average tangential velocity, which peaks at 480 m s^{-1} , as a function of radius (dashed line). Figure 3 shows a map of trajectories of test particles originally on a circle of radius 2,000 km. Indicated on the figure are the times (in minutes) required by test particles to go from the circumfer-

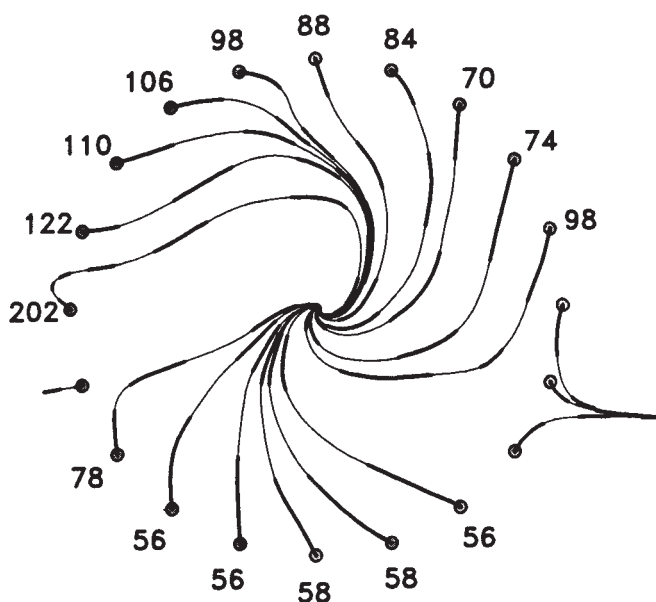


Fig. 3 The paths of test particles from the circumference of a 2,000-km radius circle centred on the vortex. The starting positions are labelled with the travel times to the centre in minutes. The path lines change between thick and thin every 15 min.

ence to the centre. The time for a particle to reach the centre of the vortex determined the average radial velocity on the trajectory. These are mostly from 600 m s^{-1} (55 min) to 270 m s^{-1} (125 min).

Figure 2 reveals that the circulation increases approximately linearly out to a radius of 1,500 km beyond which it remains nearly constant, indicating that the vertical vorticity is limited in extent. Equivalent circular areas randomly chosen in the field-of-view have circulation of $800 \pm 800 \text{ km s}^{-1}$, which is a factor of five lower than that of the core region of the vortex.

The vortex motion is obvious in the destretched time-lapse movies. Granules are seen to spiral around and disappear in the vortex centre. Some of them elongate in the streamline direction and are compressed perpendicular to the streamlines, thus resembling a solar 'maelstrom'. It is clear from the movies that all the granules in a radius less than 3,000 km from the centre of the vortex have their life histories dominated by the vortex flow. The vortex motion is definitely not an artifact created by the data reduction or the 'destretching', since it could be seen in the original data once it had been identified.

No such vortex motion has been observed previously at photospheric levels. In numerical simulations of granular convection, Nordlund¹⁸ has found that the plasma sucked into intergranular downdrafts tends to rotate around the centre of the downdraft. This 'bathtub' effect is caused by the strong density-gradient into the Sun, which concentrates downflow below the surface. The formation of prominent downdrafts, and a distinctive asymmetry between up and downflow, has also been seen in other simulations of compressible flow^{19,20}. Since any surface velocity not directed toward the sink region has a net angular momentum around the sink, vortices are likely to form. The phenomenon observed here differs from Nordlund's simulation in that it takes place on a larger, multi-granule spatial scale and is stable over many granule lifetimes.

Further investigations will be necessary to determine the frequency of these vortices and how they are related to magnetic fields. The existence of one in our small field-of-view suggests that they occur frequently as a regular element in the photospheric flow-field. Such vortical motions can, in principle, twist magnetic fields²¹ and in this way induce electric currents. Their action on twisting and interweaving the footpoints of flux-tubes

represents a mechanism for coronal heating²². Both horizontal and vertical vorticity are known to exist in turbulent fluids. In planetary atmospheres relatively stable local concentrations of vertical vorticity are not uncommon: for example, hurricanes and tornadoes on Earth and the giant red spot on Jupiter. We believe this is the first report of a stable vortex structure in the turbulent convection of the solar atmosphere.

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Pluto's structure and composition suggest origin in the solar, not a planetary, nebula

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The mean density of the Pluto-Charon system is now accurately known at $1.99 \pm 0.09 \text{ g cm}^{-3}$ (with formal errors five times smaller), through observations of total occultations and transits². Even allowing Charon's density to vary between 1 and 3 g cm^{-3} constrains Pluto's density between 1.84 and 2.14 g cm^{-3} . Pluto is thus very rock-rich, with a rock/(rock + H_2O -ice) mass ratio of ~ 0.68 – 0.80 , much greater than those of the icy satellites Ganymede, Callisto or Titan. It is so rock-rich that spontaneous unmixing of rock and ice phases in its convecting interior is just possible, and any significant differentiation during accretion or during Charon's formation, which may have involved a large-body impact³⁻⁵, renders Pluto unstable to further melting and differentiation because the resulting multiple thermal boundary layers inhibit heat transport⁶. Continual loss of methane over the age of the Solar System^{7,8} requires a near-surface methane reservoir, and implies that non-trivial differentiation has occurred⁹. Pluto is probably a differentiated object whose icy mantle is entirely in the ice-I stability field; its closest structural cousin is Europa. Of four explanations for Pluto's large rock/ice ratio¹⁰—formation in the inner Solar System, volatile loss during accretion, volatile loss during the large-body impact that created Charon, and formation as a large, ice-poor^{11,12} outer Solar System planetesimal—we show that only the last two are feasible, and that the depletion of water ice in Pluto is so severe that both explanations may be necessary.

The radii of Pluto and Charon are formally well known, at $1,122.7 \pm 3.5$ and $559.7 \pm 5.8 \text{ km}$ respectively (more realistic

Table 1 Equation-of-state parameters for the model rock types

	CI-rock	PF-rock
ρ_0 (g cm ⁻³)	2.766	3.262
α (10 ⁻⁵ K ⁻¹)	6.953	5.997
β (10 ⁻¹¹ Pa ⁻¹)	1.878	1.479

According to the form $\rho(P, T) = \rho_0[1 - \alpha(T - T_0) + \beta(P - P_0)]$, where T_0 and P_0 are standard temperature and pressure (STP).

errors being ± 20 km for both)¹. The primary systematic uncertainty is due to Charon's orbital radius (the semimajor axis of the reduced system) (M. Buie, personal communication). The planet and satellite radii scale as the semimajor axis, so the total volume and (by Kepler's third law) the total mass of the system scale as the cube. Thus the mean density is insensitive to these changes. The individual masses of Pluto and Charon are not yet known, but the generous range in Charon's density mentioned above, bracketing all known values for icy satellites, provides useful bounds on that of Pluto. Although similar in density to Ganymede (1.94 g cm⁻³), Callisto (1.86 g cm⁻³) and Titan (1.881 g cm⁻³), Pluto is not like these objects, which are substantially self-compressed. It is a new kind of world, intermediate in size between the largest medium-sized icy satellite, Titania (800-km radius), and the smallest icy Galilean satellite, Europa (1,569 km)¹³.

We build structural models for Pluto as we did previously for Ganymede and Callisto⁶. We explore variation with temperature, the minimum and maximum density limits, differentiation state and rock mineralogy. Rather than simply choosing plausible density values for the rock fraction, we use two mineralogical models for rock components of outer Solar System objects, representing two distinct degrees of hydration and oxidation⁶. The first is a mineralogical analogue to CI carbonaceous chondrite, the most volatile-rich type (hereafter CI-rock), in which Mg, Fe, S and Ca are constrained to their Si-normalized CI abundances. The second is a theoretical condensate derived by Prinn and Fegley¹⁴ (hereafter PF-rock), an equilibrium assemblage with solar abundances and water content intermediate between that of CI-rock and completely anhydrous rock (we do not model the latter for reasons below). Equation-of-state (EOS) parameters are given in Table 1. The CI-rock density is high because it does not include porosity or the ~ 10 wt% organic-rich 'residue' of CI chondrites¹⁵; we treat this latter as part of the ice component, obeying the ice EOS.

The variability in the models is simplified by correlating differentiation state with rock mineralogy. CI-rock is used in undifferentiated models and PF-rock in differentiated ones, reflecting the probable thermal metamorphism and partial dehydration of relatively large silicate cores (the core sizes derived here exceed the size of any known asteroid). Silicates accreted in the solar nebula may be largely anhydrous, because hydration reactions proceed quite slowly over the lifetime of the nebula¹⁶. Nevertheless, we hypothesize that accretion and burial in intimate contact with warm water ice (as in an undifferentiated Pluto) allows silicates and other rock-forming minerals to hydrate fully over the age of the solar system. Similarly, we do not model anhydrous rock cores, because cores form by melting (of the ice fraction) and differentiation, leading inevitably to hydration. A core in the size range derived here is not large enough for its bulk to be heated radiogenically to the temperatures necessary for total dehydration (~ 800 – $1,200$ K, ref. 17).

The first suite of models takes the ice component to be solely water-ice. Temperatures are chosen to represent two extremes: 'hot' models possess core temperatures of 1,000 K, and ice or mixed ice-rock temperatures are always 5 K less than the ice melting temperature at the appropriate pressure; 'cold' models are 130 K everywhere. The temperature dependence of the

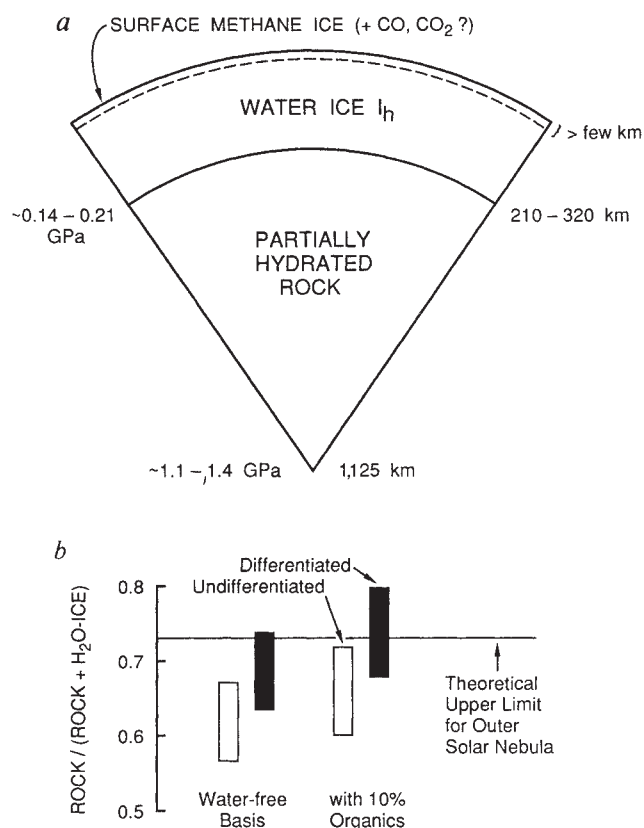


Fig. 1 *a*, Cross-section of Pluto's present-day structure, inferred to be differentiated. The base of the mantle may also contain an organic layer and, if Pluto's density is low enough, a thin mixed ice-I-ice-II layer. *b*, Rock/(rock + H₂O-ice) mass ratios under various circumstances, compared to the theoretical upper limit for a CO-dominated nebula. The two ratios at right are calculated as if the rock is water-free as well, but include organics at 10% of the rock mass fraction.

results is small. Considering the total range in all the model parameters, an undifferentiated Pluto should have an ice-VI center and a central pressure of 0.6–0.9 GPa. A differentiated Pluto should have a higher central pressure, 1.1–1.4 GPa, a large silicate core, and a pure ice-I mantle 200–300 km thick (Fig. 1). Cold models actually have ice-II at the bases of their mantles, but realistic thermal profiles admit at most a thin (<25 km) mixed ice I-II layer at the bottom of a convection zone, and this only for Pluto densities less than the system density. Differentiation causes an increase in radius of $\sim 4\%$.

Perhaps our most important results are estimates of Pluto's rock fraction or, more specifically, the rock/(rock + H₂O-ice) mass ratio. These range from 0.67–0.79 for undifferentiated models with the minimum and maximum density, expressed in terms of CI-rock, to 0.68–0.79 for differentiated models, expressed in terms of PF-rock. Variability related to temperature is about ± 0.01 for differentiated models and about ± 0.02 for differentiated ones (the latter is a result of the temperature sensitivity of the ice-I-ice-II transition pressure). Variability due to differentiation state (coupled to mineralogy) is clearly smaller.

Not only are these rock mass ratios high, but the corresponding rock volume fractions for the undifferentiated models range between 0.40 and 0.56, just below the $\sim 60\%$ necessary for breakdown of convective self-regulation of Pluto's internal temperature¹⁸. At this point silicate particles form a rigid interconnected framework and control of viscosity by ice is no longer possible; temperatures rise past the ice melting point and differentiation ensues. Although it is not certain that an undifferentiated Pluto must melt down, if Pluto underwent even

minimal differentiation early in its history, then complete differentiation is likely to follow. Partial differentiation leads to a three-layer structure (rock-rich core or central region, mixed rock-ice lower mantle and ice-rich surface region or upper mantle). The upper two icy regions will convect separately and the resulting multiple thermal boundary layers cause the temperature in the mixed region to exceed the water-ice minimum melting temperature of 251 K; in Pluto's case the top of the mixed region is always in the ice-I field owing to its temperature. Thermal profiles can be constructed for Pluto's convecting regions using adiabats and estimating the temperature increase across each thermal boundary layer by evaluating the Rayleigh number of each boundary layer, $Ra_{bi} = g\alpha\rho k^3(\Delta T)/\eta\kappa F^3$ (ref. 19). ΔT and F are the temperature difference across and heat flux through a boundary layer, and g , α , ρ , η , k and κ are gravity, thermal expansion coefficient, density, viscosity, thermal conductivity and thermal diffusivity. Appropriate values⁶ are used to evaluate Ra_{bi} 4 Gyr ago. In particular, the viscosity chosen is the smallest from experimentally determined power-law and theoretical diffusive rheologies; this minimizes the likelihood of melting⁶. Usually Ra_{bi} is considered to be approximately 10^3 (ref. 19). In this case, 251 K is reached easily within Pluto and differentiation results. A less prejudicial approach determines the 'critical' boundary-layer Rayleigh number for which the melting curve is intersected. For Pluto this critical value is ~ 10 –20. Melting and differentiation are likely.

Pluto's widespread (but not ubiquitous) surface methane layer^{20–22} is evidence for partial⁹ and thus, by the argument above, global differentiation. Estimates of hydrodynamic escape of methane over the age of the Solar System range from a few to ~ 20 km (refs 7, 8). This requires a substantial near-surface reservoir of methane (~ 0.2 –1.5% of Pluto's mass). If Pluto formed in the outer, CO-dominated¹¹, solar nebula, then release of enclathrated methane (at $\sim 3\%$ or less of the water abundance^{23–25}) can supply the required amount. Because clathrate is stable (in the absence of major NH_3) to greater than 1 GPa (ref. 23), substantial melting of water ice ($>1/3$ by mass), and hence differentiation, is implied. Alternatively, CH_4 may be supplied by reduction of organic matter and graphite in a core. Here a large enough core region must reach cracking temperatures of ~ 650 K (refs 26, 27). For plausible conductive heating by U, Th and K decay, the minimum core size exceeds 600 km in radius, again implying major differentiation ($>1/3$ by mass).

A comparison with Ganymede and Callisto is instructive. The uncompressed density of a differentiated Pluto is within one per cent of the bulk estimates. For differentiated models with PF-rock cores, the maximum values for Callisto and Ganymede are significantly smaller, ~ 1.53 – 1.58 g cm⁻³ (ref. 6). Unless Pluto is undifferentiated and close to minimum density, it is considerably enriched in silicates compared to all icy satellites save Europa.

The estimates of rock fraction can be refined further, as organic matter is counted as part of the ice fraction (which is appropriate for calculating volume fractions that affect viscosity or uncompressed density). For models containing organics at 10% of the rock mass fraction, the adjusted rock fractions are ~ 0.72 – 0.86 for both differentiated and undifferentiated models. CI-rock contains 15.7% and PF-rock 6.1% H_2O . On a water-free basis, the rock mass fractions become 0.60–0.72 for undifferentiated models and 0.68–0.80 for differentiated ones (Fig. 1). For the probably differentiated case, the effects on the rock/ice ratio of accounting for bound water and organics compensate. In addition, adding a cool (50 K) low-density surface methane layer (EOS from ref. 28) to the models drives the rock/(rock + water-ice) mass fraction up by about 1.7% per 10 km of methane for fully differentiated models and 3.1% per 10 km for undifferentiated ones. Clathration of water ice increases the rock mass fraction as well, unless (for differentiated models) the guest is of greater molecular weight than CO. Clearly, Pluto's high rock/ice ratio is a fundamental clue to its origin. We consider four explanations.

Pluto formed in the inner Solar System: this proposal goes back to erroneous ideas that Pluto is made of impossibly dense materials, but does not explain how Pluto acquired its non-negligible ice component and has acquired a satellite. It is not impossible but extremely unlikely²⁹ that an object would be scattered into the outer Solar System only to be trapped in a resonance with Neptune while there are plenty of local objects to be captured. Similar difficulties apply to Pluto's originating as a satellite close to Jupiter (in the Io-Europa region).

Pluto lost volatiles during accretion: Ganymede, Callisto and Titan may have lost water ice preferentially through impact vaporization³⁰, thereby increasing satellite rock/ H_2O -ice ratios from the $\sim 40/60$ cosmic (or equilibrium) value characteristic of the middle-sized satellites of Saturn^{30–32}. This mechanism fails for Pluto, however, as accretional velocities are low (≤ 1 km s⁻¹; see Fig. 9 in ref. 30). The presence of protosatellite nebular gas, relevant to scenarios where Pluto originates as a satellite of Uranus or Neptune, is unlikely to affect the situation by allowing water vapour to flow back into the nebula³³. It is still the gravitational potential energy of the forming body that provides the heating for vaporization, insufficient in this case (J. Lunine, personal communication).

Pluto lost volatiles during the large-body impact that created Charon: such a collision would be relatively slow (the combined escape and out-of-ecliptic velocity is ~ 2.5 km s⁻¹), so most debris is likely to be retained. A sufficiently oblique collision could lead to the formation of a re-assembled binary, as has been suggested for some asteroids (refs 34, 35). Pluto's angular momentum density and obliquity could be explained. Collision with a differentiated proto-Pluto might lead to permanent loss of icy debris (as opposed to simply forming an ice-rich Charon) because of the finite size of Pluto-Charon's resonant (with respect to Neptune) orbital phase space, but this remains to be carefully evaluated. Such an impact model is consistent with and may be necessary for entry into the resonance. In other words, we would be hard pressed to account for Pluto as the sole occupant of the resonance with Neptune. A binary with such a large secondary: primary mass ratio ($\sim 1:7$) is a testament to a significant change in orbital angular momentum. Indeed, we might ask whether Pluto-Charon is simply the largest member of family of objects sharing the resonance.

Pluto is a large outer Solar System planetesimal: this is dynamically plausible and provides a natural explanation in terms of nebular chemistry³⁶. Kinetic inhibition of equilibrium in the outer solar nebula favors the production or maintenance of molecular nitrogen and oxidized carbon compounds at the expense of ammonia and methane¹¹; many people have discussed the effect on the rock/ H_2O -ice ratio^{12,16,32,37}. Essentially, if carbon is in the form of CO, enough oxygen is taken up to raise the rock/ice ratio. Summing the abundances³⁸ of Si, Fe, Ni, Mg, Al, Ca, S and Cr with an O_4 for every Si gives 73% rock, 27% ice, if all carbon is in the form of CO. This matches the mid-range of the values for a differentiated Pluto (Fig. 1) but, because some carbon must be in the form of organics (or graphite) and surface methane, 0.73 is an upper limit for the rock mass fraction. There remain considerable uncertainties in the C and O abundances³⁸, but Pluto's rock/ice ratio appears so high that both formation in a CO-dominated nebula and large-body impact may need to be invoked. Formation in the cool regions of any planetary nebula resembling that which gave birth to the Uranian, Saturnian, or outer Galilean satellites, with their much higher ice contents, is ruled out. We also note that the presence of CH_4 on Pluto's surface is not an argument against CO dominance; minor CH_4 will be strongly partitioned into clathrate as argued for Halley²⁵ and, as noted above, methane can be produced in Pluto's core. CO should be searched for spectroscopically, however, and its detection, although difficult in the near-infrared, is an important test of the conclusions advanced here. Similarly, the rock/ice ratio of Triton should be determined by Voyager 2 when it reaches Neptune,

and it will bear critically on the hypothesis that Triton was captured from solar orbit (see, for example, ref. 3).

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Note added in proof: A preprint by Simonelli *et al.* on the carbon budget of the outer Solar System includes Pluto models whose rock fractions are consistent with ours.

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The effect of hydrogen on oxygen diffusion in quartz: evidence for fast proton transients?

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The importance of water in modifying the physico-chemical and mechanical properties of minerals has long been recognized. Water dramatically weakens quartz and enhances oxygen diffusion in silicates at high pressures and temperatures, with important geological consequences, but the underlying mechanisms are poorly understood. Recent attention has focused on the role of H₂ and O₂ in these mechanisms. Here we report the results of a study of the diffusion of oxygen in quartz at high pressure and temperature in the presence of water and of dry H₂ gas, and compare the results with those of experiments in the presence of dry O₂ gas. We conclude that oxygen diffusion is inhibited in the presence of dry H₂ but enhanced in the presence of H₂O, and deduce that fast H⁺ transients rather than H₂ movement may be responsible for oxygen diffusion enhancement in framework silicates. Because of their high mobility, such transients may well be experimentally unquenchable within the silicate framework.

Water enhances mineral reactivity through solution-precipitation processes¹, greatly increases Al/Si interdiffusion rates in feldspars²⁻⁴, influences the conductivity of quartz⁵, and causes dramatic 'hydrolytic' weakening of quartz in deformation experiments and in nature^{6,7}. Oxygen diffusion in quartz and feldspars at high temperatures under 'dry' conditions is characterized by high activation energies and slow diffusion rates compared with oxygen diffusion in these minerals under 'wet' (hydrothermal) conditions, where low activation energies and fast diffusion rates prevail⁸⁻¹² (Table 1). These observations place important constraints on the closure temperatures and processes for cessation of diffusive oxygen exchange in cooling crustal metamorphic or hydrothermal rocks, and therefore on the interpretation of oxygen isotopic compositions of minerals used in studies of fluid-rock interaction and in geothermometry¹³⁻¹⁶. They also demonstrate the involvement of a hydrogen-bearing species in the mechanism of enhancement of

oxygen diffusion in framework silicates. However, in spite of much recent research, water enhancement mechanisms for intracrystalline processes in general and for oxygen diffusion in particular, and the role of the grain-boundary fluid in these processes, are poorly understood. In experimental and theoretical studies emphasis has tended to focus on the role of H₂ and O₂ in diffusion and deformation processes in quartz and feldspar^{9,17-22}.

'Water' is known to occur in trace amounts in natural and synthetic quartz. The solubility of hydrogen in natural quartz is of the order of 100 H/10⁶ Si, occurring interstitially, with charge-compensating Al (or Fe) replacing Si in the lattice^{23,24}. The dominant hydrogen impurity in synthetic quartz, at concentrations of thousands of p.p.m. (H/Si), is molecular water, which is associated with the hydrolytic weakening process²⁵. Water-related defects and their mobilities are known from infra-red studies of quenched samples.

In an effort to clarify the hydrogen species involved in the enhancement of oxygen diffusion in quartz, we have conducted annealing experiments on quartz in the presence, in turn, of H₂O and then of dry H₂ at high pressure and temperature. A polished (001) section of M3 quartz⁹ was annealed in a cold-seal hydrothermal bomb for 153,600 s (~42 h) in a sealed Pt capsule at 700 °C and 1 kbar in ¹⁸O enriched water (20 at.%). Capsule fugacity of oxygen, *f*_{O₂}, was controlled by the bomb near the NNO buffer. This first anneal established an ¹⁸O diffusion profile in the near-surface region of the quartz¹¹. The sample was then sawn into two pieces and one half re-annealed in hydrogen. For this latter part of the experiment the quartz sample was placed

Table 1 Comparison of oxygen diffusion constants for two framework silicates under 'wet' versus 'dry' conditions at high pressure and temperature

$D = D_0 \exp(-Q/RT)$ Units: D_0 (cm ² s ⁻¹) Q (kcal mol ⁻¹)			
	D_0	Q	
Anorthite (CaAl ₂ Si ₂ O ₈)			
'wet'	2×10^{-7}	26	1 kbar, 400-600 °C (ref. 8)
'dry'	9×10^{-6}	56	1 bar, 800-1300 °C (ref. 12)
Quartz (SiO ₂)			
'wet'	2×10^{-7}	33	1 kbar, 600-850 °C (ref. 9)
'dry'	3×10^{-7}	53	1 bar, 600-850 °C (ref. 10)

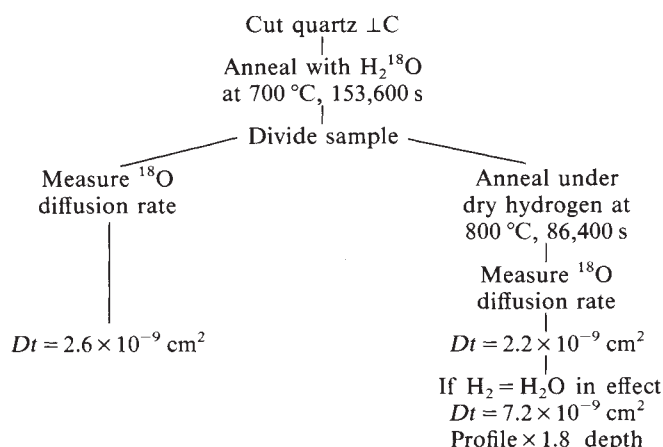


Fig. 1 Summary of experimental conditions, rationale and results.

in a gold capsule, previously welded shut at one end. The capsule was then crimped at the other end, and placed in a second platinum capsule, which was welded shut at one end but left loosely crimped at the other. Silicon metal powder was packed around the inner capsule as an oxygen getter. The double capsule was loaded into an internally heated pressure vessel which has been specially modified to run at high hydrogen pressures, and was run for 86,400 s (24 h) at 800 °C and 1 kbar in an atmosphere of 1:1 hydrogen/argon. This gas mixture was in direct communication with the quartz through the open-ended gold capsule, but was dried, during diffusion through to the quartz, by passage over the powdered silicon. Thus the quartz was bathed in pressurized dry hydrogen gas.

Ion microprobe (SIMS) profiles for ^{18}O concentration were measured for the two samples and recalculated as reciprocal error function plots in the normal way¹¹. Both gave the expected linear relationships, with plot gradients giving $(2\sqrt{Dt})$, from which D is readily derived if t , the effective diffusion anneal time, is known. The experimental results are shown in Fig. 1, which summarizes the SIMS results for the two ^{18}O profiles generated. The initial profile formed by the primary hydrothermal anneal gave $D = 1.7 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$, derived from a Dt value of $2.6 \times 10^{-9} \text{ cm}^2$. After annealing in hydrogen the measured ^{18}O profile in the second half of the quartz sample was identical to that in the first (within analytical error), recording a Dt value of $2.2 \times 10^{-9} \text{ cm}^2$. The D value derived from the first anneal matches the values to be expected from a single hydrothermal anneal¹¹ ($D = 1.8 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$). The conclusion is that hydrogen gas does not enhance oxygen movement in quartz in the same way that 'water' has been demonstrated to do. If it had done so in the experiment outlined above, the final Dt value at the end of the second double anneal should have been $7.2 \times 10^{-9} \text{ cm}^2$ (ref. 11), implying an ^{18}O penetration profile 1.8 times longer than that actually measured. A duplicate experiment at 800 °C, 1.5 kbar, 2:1 H_2/Ar , gave identical results.

In summary, the presence of H_2O as the external fluid enhances oxygen diffusion in quartz and feldspar, in contrast to anneals using O_2 or H_2 media. The experiments reported above imply that any active species mediating this behaviour is either local to the lattice or moves at much higher velocities than does oxygen. This has been demonstrated by causing the same piece of quartz to exhibit both 'fast' and 'slow' oxygen diffusion.

Two simple possible mechanisms can be advanced to explain the reported observations. The first possibility is that mere contact with H_2 changes the defect chemistry or electronic state of the silicate lattice, without movement of a defect species into the structure. However, existing oxygen diffusion data for framework silicates, summarized in Table 1, show little evidence for fundamental changes in diffusion mechanism. A simple vacancy mechanism is generally invoked for oxygen diffusion

in silicates, and the restricted range in the pre-exponential factor (D_0) in Table 1 argues for there being no major change in vacancy concentration between 'wet' and 'dry' environments. The systematic changes in activation energy (Q) suggest that it is the energetics of the diffusion jump process which are affected by the presence of 'water'.

The second possibility is that there is an atomic or molecular species present in H_2O , but not in O_2 or H_2 , capable of penetrating framework silicates at high velocity and of modifying lattice behaviour in a homogeneous manner. The obvious and simplest candidate is H^+ , which could alter the local environment of oxygen vacancies, making diffusion energetically easier. A mechanism involving 'proton-activated transient coordination increase' with Si-O and Al-O bond breaking has been advanced to explain enhanced high-pressure disordering in feldspars²⁶, and the Frank-Griggs mechanism⁷ involving Si-O bond hydrolysis is well studied in rock deformation experiments.

In the diffusion experiment reported above, H_2O would provide a higher concentration of H^+ than would H_2 —especially at high pressures, where H_2O dissociation is extensive²⁷⁻²⁹. Thus, relative to the mineral oxygen lattice, H_2O would be an H^+ source whereas O_2 or H_2 would be an H^+ sink. The contention that H_2 can act as a process inhibitor is supported by the study by Ord and Hobbs²² of hydrolytic weakening of quartz. The strength of quartz was measured under fixed pressure/temperature conditions across a wide range of H_2 values, to extremes of high fugacity of H_2 , f_{H_2} . Their results show that natural single-crystal quartz remained strong at high f_{H_2} (low $f_{\text{H}_2\text{O}}$), but weakened at high $f_{\text{H}_2\text{O}}$ (low f_{H_2}).

As summarized at the beginning of this letter, H_2O has an extreme effect on many geological processes. However, it appears that the variable of prime importance in understanding some mineralogical changes affected by H_2O is not f_{O_2} or f_{H_2} , as is often thought, but the activity of H^+ ions, a_{H^+} . Thus geological fluids should be examined for their ability to supply or maintain H^+ on a mineral lattice during solid-state changes. Any process that affects H^+ concentration in the fluid, such as salt solution or pressure/temperature changes, can be of great importance.

In conclusion, the rapid change in oxygen diffusion rates in quartz which we report can be explained by appeal to fast proton transients through the framework silicate lattice. The lack of hysteresis effects suggest that such transients are unquenchable, that they may be undetectable except by observation at experimental pressures and temperatures, and that they may differ from hydrogen species identified in quenched quartz samples.

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Satellite detection of transient enhanced primary production in the western Mediterranean Sea

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Primary production by marine microalgae is believed to be a critical factor regulating atmospheric carbon dioxide levels and associated climatic changes¹. Assessments of photosynthesis in the open ocean^{2–4}, and the related export of organic carbon to the deep ocean (new or non-regenerative production^{5–7}, vary by as much as an order of magnitude (refs 2 and 6, compare with refs 3–5 and 7). Discrepancies are attributed^{4,6–8} to different temporal and spatial scales reflected by instantaneous rate measurements, as opposed to seasonally averaged measurements based on subsurface changes in chemical tracers. Satellite extrapolations of primary production can be used to characterize and quantify temporal and spatial variability^{9–11}. But time differentials between satellite and ship measurements, as well as regional and seasonal variations in empirical relationships, have so far limited the precision of such extrapolations^{9,12}. We conducted extensive ship sampling of chlorophyll *a* and primary production in the western Mediterranean Sea contemporaneous with Nimbus-7 coastal zone colour scanner imagery. Our approach resulted in an empirical model for estimating integrated water-column primary production from satellite imagery. Precision was adequate to resolve short-term fluctuations in primary production associated with a meso-scale circulation feature.

Sampling was conducted from 5 May to 21 May 1986 aboard USNS *Lynch*, encompassing wide variations in weather and water mass characteristics. Vertical stations (Fig. 1a) transected density fronts that exist as a consequence of juxtaposition of Mediterranean Sea water and less saline Atlantic water entering through the Strait of Gibraltar. This Modified Atlantic Water becomes entrained in twin anticyclonic gyres in the Alboran Sea (ref. 13; R.A.A., D.A.W. and K. D. Saunders, manuscript in preparation). The flow exits the second gyre near 1° W longitude and branches eastward to form the Algerian Current^{14,15}. Strong density fronts persist between Modified Atlantic Water and Mediterranean Water as far as 3° E longitude.

We examined the synoptic distribution of primary production by correlating satellite-derived surface pigment concentrations, and shipboard measurements of surface chlorophyll *a* and integrated water-column primary production. Coastal zone colour scanner (CZCS) imagery was processed^{16,17} to yield surface photosynthetic pigment concentrations (C_k in mg pigments m^{-3}) using a single scattering Rayleigh model and a mid-latitude ozone absorption coefficient. The 670-nm channel was used for aerosol path removal with an epsilon value of

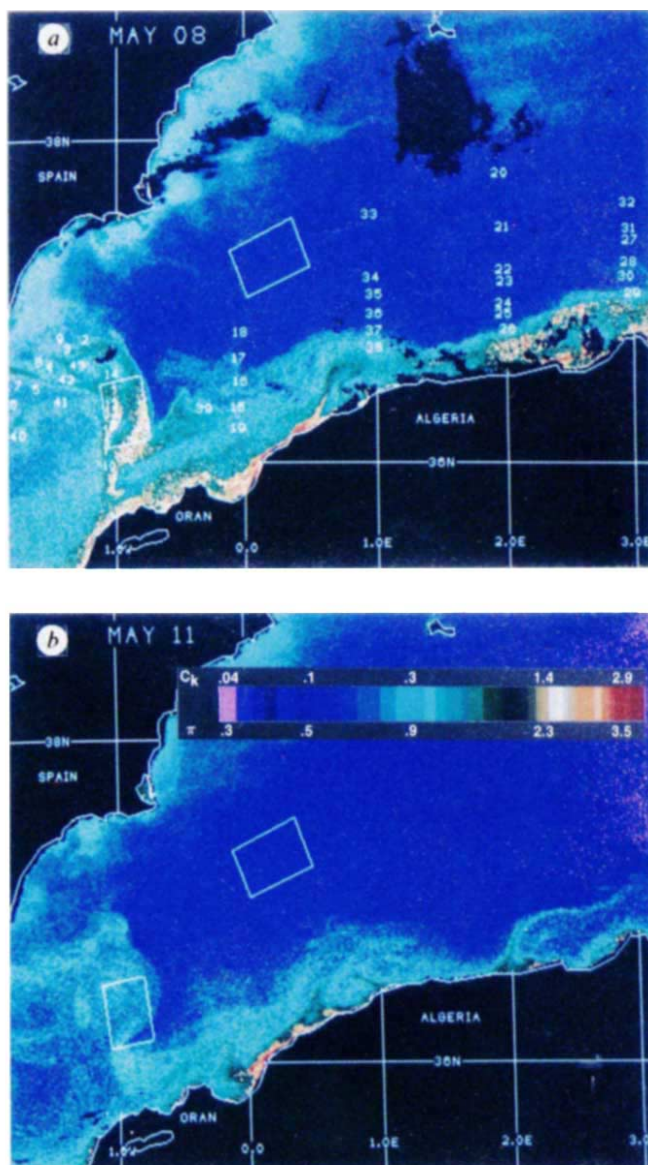


Fig. 1 CZCS images from 8 May (a) and 11 May (b) showing distribution of near-surface photosynthetic pigment concentrations (C_k in mg pigments m^{-3}) and integrated primary production (π in $g\ C\ m^{-2}\ d^{-1}$). The images have been resampled to a mercator projection such that each pixel represents $1\ km^2$. The order and location of ship stations are indicated by numbers in a. The colour scale in b applies to both images. Statistics in Table 1 were determined for image areas representative of the Modified Atlantic Water current (western box) and Mediterranean Water (eastern box).

0.990 for the 550-nm channel. Ship estimates of surface chlorophyll *a* (C_s in mg pigments m^{-3}) were made on samples collected roughly every 6 km while the ship was underway. Samples were also collected at 5-m intervals to a depth of 100 m at the vertical stations (Fig. 1a). Concentrations were determined in duplicate using modifications of the methods of Smith *et al.*¹⁸, as described by Lohrenz *et al.*¹³. Water-column primary production (π in $g\ carbon\ (C)\ m^{-2}\ d^{-1}$) at the vertical stations was estimated from vertical profiles of chlorophyll *a* using an empirically determined relationship between chlorophyll-specific ¹⁴C-primary production and depths¹³.

The relationship of water-column production (π) and surface chlorophyll *a* (C_s) at the vertical stations (Fig. 2a) was highly significant ($r^2 = 0.758$, $N = 39$). To develop a predictive equation for satellite extrapolation of π , individual pixel values of C_k

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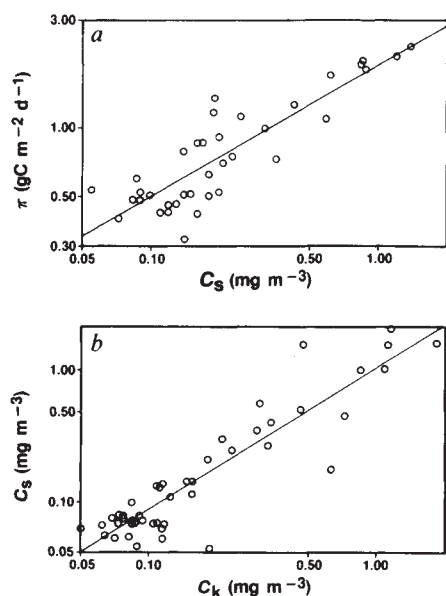


Fig. 2 *a*, log-log relationship of surface chlorophyll *a* concentration (C_s) versus integrated water-column primary production (π) determined at vertical stations. A model II regression²² yields the equation: $\ln(\pi) = 0.633 (\pm 0.145, 95\% \text{ confidence interval}) + 0.579 (\pm 0.090) \ln(C_s)$ (solid line). The r^2 of the correlation was 0.758 ($N = 39$). *b*, log-log relationship of CZCS-derived near-surface photosynthetic pigment concentrations (C_k) versus coincident surface chlorophyll *a* concentrations (C_s) determined from underway measurements. A model II regression gives the equation: $\ln(C_k) = 0.001 (\pm 0.190) + 0.994 (\pm 0.110) \ln(C_s)$ (solid line). The r^2 of the correlation was 0.841 ($N = 48$).

corresponding to the ship's latitude and longitude were extracted from five CZCS images (5, 8, 10, 11 and 12 May) and compared with values of C_s determined within 8 h of the satellite passing (Fig. 2*b*). The C_k versus C_s relationship was significant ($r^2 = 0.841$, $N = 48$) with a slope of 0.994 and an intercept not significantly different from zero. Although there was a persistent subsurface chlorophyll maximum¹³, it occurred below the depths detected by satellite. The agreement we observed over both time and space between C_s and C_k indicates that atmospheric corrections applied to CZCS data were accurate.

A predictive model for extrapolating integrated water-column production from satellite-derived pigments (C_k) was developed by substituting the C_s term in the π versus C_s relation (Fig. 2*a*) with the equation for estimating C_s from C_k (Fig. 2*b*). The resulting relationship between π and C_k (Fig. 3) is described by the equation: $\ln(\pi) = 0.634 + 0.576 \ln(C_k)$. π and C_s data from Fig. 2*a* are replotted in Fig. 3 to illustrate the fit. Because of the extent of our ship data and its close relationship to the satellite imagery, the precision of CZCS extrapolations of π in our study is significantly improved over that of previous investigations⁹⁻¹².

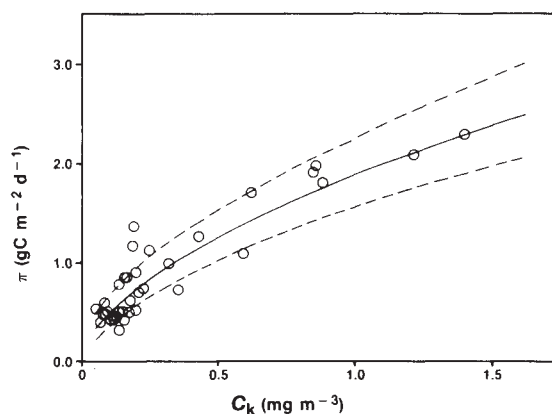


Fig. 3 Predicted integrated water-column primary production (π) based on CZCS-derived near-surface photosynthetic pigment concentrations (C_k). The predictive equation resulting from combining regression models shown in Fig. 2*a* and *b* is $\ln(\pi) = 0.634 + \ln(C_k) 0.576$ (solid line). Dotted lines show 95% confidence limits of the prediction, propagated from errors of regressions in Fig. 2. Symbols represent the same data shown in Fig. 2*a* but replotted for comparison.

We used the predictive model in Fig. 3 to extrapolate π from time-series CZCS data. CZCS images from 8 May and 11 May (Fig. 1*a* and *b* respectively) illustrate areas of higher near-surface pigment concentrations and integrated primary production associated with a current of Modified Atlantic Water (MAW) in the western Mediterranean Sea (ref. 13; R.A.A., D.A.W. and K. D. Saunders, manuscript in preparation). The dramatic decrease in both pigment concentration and primary production from 8 May to 11 May illustrates their transient nature in that current. To quantify these changes, we selected regions on the CZCS images that were representative of relatively high (MAW, western box in Fig. 1*a* and *b*) and low (Mediterranean Water, eastern box) pigment and production levels. The statistics for the boxes are shown in Table 1 for CZCS images obtained between 30 April and 17 May 1986. In the MAW, relatively high values of π ($1.8 \text{ g C m}^{-2} \text{ d}^{-1}$) and C_k ($0.9 \text{ mg pigment m}^{-3}$) occurred on 8 May, followed by a rapid decline. Levels increased again on 17 May. π and C_k were consistently lower for the Mediterranean Water.

The satellite interpretations provide evidence that a substantial fraction of regional production is attributable to localized and highly variable primary production events. The fronts between MAW and Mediterranean Water are sites of enhanced vertical mixing^{13,19}. As nitrate concentrations were higher at depth^{13,19}, nitrate enrichment could thus explain the enhanced primary production observed in the MAW current, leading to the conclusion that a significant proportion of total production is new production in that area.

Recognition and characterization of transient primary production events in other nutrient-limited regimes may help to

Table 1 Time series of satellite-estimated integrated water-column primary production (π) and near-surface pigments (C_k)

Date	Modified Atlantic Water			Mediterranean Water		
	π ($\text{g C m}^{-2} \text{ d}^{-1}$)	C_k (mg m^{-3})	CV (%)	π ($\text{g C m}^{-2} \text{ d}^{-1}$)	C_k (mg m^{-3})	CV (%)
30 April	1.2	0.45	15	0.5	0.11	21
8 May	1.8	0.90	19	0.6	0.13	14
11 May	0.8	0.25	14	0.5	0.11	16
12 May	0.6	0.15	17	0.3	0.05	91
17 May	1.9	1.10	15	—	clouds	—

Estimates were determined for areas representative of the Modified Atlantic Water current and Mediterranean Water (western and eastern boxes in Fig. 1 respectively). Means and coefficients of variation (CV) were determined for pixel values before converting to π and C_k . Area in km^2 (and number of pixels) was 1,996 for modified Atlantic Water and 2,141 for Mediterranean Water boxes. Representative attenuation lengths were determined using a spectral radiometer²¹ and were 15 m for Mediterranean and 13 m for Modified Atlantic Water types.

resolve discrepancies between integrated synoptic-scale tracer measurements and discrete instantaneous rate measurements. Jenkins⁷ suggested that a major source of nitrate flux in the North Atlantic Ocean may result from nutrient injection events associated with mesoscale features. Whereas subsurface tracer measurements^{3-5,7} must infer such pulses from long-term averaged data, satellite imagery provides the ability to examine transients in near real-time. Closer coordination of upper ocean measurements and remote sensing research²⁰ should lead to more precise estimates of primary production and an improved understanding of contributions of marine biogeochemical cycles to global scale processes.

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Quantitative analysis of radiocaesium retention in soils

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The fallout of radiocaesium after the Chernobyl accident has renewed interest in its environmental behaviour. How it behaves in soils and sediments is important, for example, for the modelling of radiocaesium transport and retention in soils, and transfer from soil to plants and hence into the food chain. The traditional approach is highly empirical and is based on the measurement of solid-liquid distribution coefficients (K_D values) and transfer factors. It is generally believed that radiocaesium retention in soils and sediments is due to the presence of a small number of highly selective sites. Neither their abundance nor their Cs-selectivity has been quantitatively determined. Here we report a new methodology which achieves such characterization. Previously studies of radiocaesium in soils have foundered because K_D values have been derived under conditions very different from those *in situ*. We show that *in situ* K_D values can be predicted from readily measurable

Table 1 Properties of the four soils

	CEC (meq kg ⁻¹)	Granulometric data (%)				% Organic carbon
		Clay (<2 µm)	Loam (2-20 µm)	Loam (20-50 µm)	Sand (>50 µm)	
Soil 1	71	8.4	3.5	6.5	81.6	0.1
Soil 2	144	21.8	2.0	4.2	72.0	0.1
Soil 3	161	41.1	1.6	3.6	53.7	0.2
Soil 4	364	35	21.5	27.1	16.4	1.2

soil properties, thus enabling information about the mobility of radiocaesium in soils to be reliably and easily obtained. These findings can be generally applied to a wide variety of soils.

From the research in weapons fallout conducted in the 1960s and 1970s (ref. 1), there emerged a general consensus that the retention behaviour of radiocaesium in soils and sediments is essentially controlled by illite (hydrous mica), the dominant clay mineral in the soils of Western Europe. Direct evidence has shown that fallout radiocaesium is associated to a very significant extent with micaceous clays^{2,3}. In particular it appears that trace levels of poorly hydrated alkali cations such as K⁺, Rb⁺ and Cs⁺ are retained very selectively at a number of specific sites, located at the frayed edges of the clay particles⁴. The very strong retention of Cs⁺ on soil and sediment particles is well illustrated in the extensive literature on the use of fallout radiocaesium in soil-erosion and sediment-deposition studies⁵.

We have recently shown⁶ that the sorption of caesium in illite clay can be described in terms of three kinds of sites, two of which are probably located on the clay crystal edges and which contribute ~2% of the overall cation-exchange capacity. One of these sites was shown to exhibit an exceedingly high Cs⁺ to K⁺ selectivity. Recently, we have introduced some significant methodological developments that allow a quantitative assessment of both the number and the selectivity pattern of such specific sites in soils. The method is based on the use of the silver thiourea (AgTU) complex to mask the bulk of the ion-exchange complex of the clay, specifically the regular ('planar') ion-exchange sites. AgTU has been shown to be very selectively retained on ion-exchange sites^{7,8} and has been proposed as an index cation for measuring cation-exchange capacities (CEC) of soils, irrespective of organic carbon content⁹. Thus, by dispersing a soil in a solution containing suitable background levels of AgTU, caesium ions are excluded from the bulk of the ion-exchange complex and their action becomes restricted to the small number of specific sites that are inaccessible—either for steric or selectivity reasons—to the bulky AgTU complex. Caesium sorption isotherms can be measured for these Cs-selective sites.

Here we describe the methodology and the results obtained on four soils of widely varying textural properties and exchange capacities. Soil properties are summarized in Table 1. Soil samples are dispersed in a solution of 0.015 M AgTU and centrifuged, and the supernatants discarded. Cs sorption isotherms are measured by equilibrating the AgTU-saturated soil samples with caesium solutions, labelled with ¹³⁷Cs, of varying initial concentrations in a background concentration of 0.015 M AgTU, and monitoring the activity of ¹³⁷Cs in the liquid phase before and after addition of the samples.

Sorption isotherms are shown in Fig. 1. Sorption plateaus are observed at ~1-4 meq kg⁻¹ soil; plateau values provide an estimate of the relative numbers of frayed-edge sites (FES). There is a minor contribution from Cs adsorbed on planar and organic-matter exchange sites, for which a correction can be applied on the basis of AgTU-Cs selectivity studies on the planar exchange sites in illite. Our measurements indicate a value of ~45 for the selectivity coefficient K_c , defined as

$$K_c = \frac{Z_{\text{AgTU}} m_{\text{Cs}}}{Z_{\text{Cs}} m_{\text{AgTU}}} \quad (1)$$

where Z and m refer, respectively, to the relative occupancy of

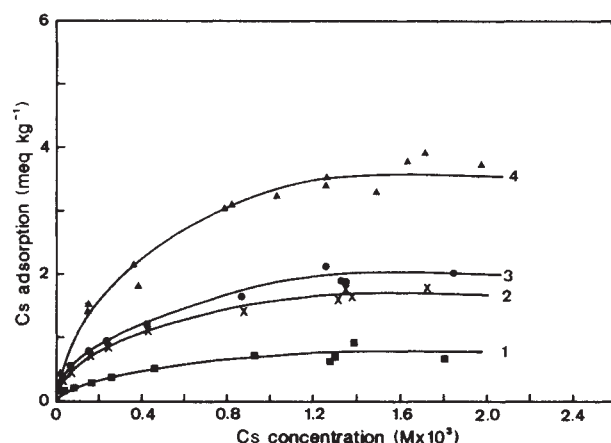


Fig. 1 Sorption isotherms of caesium in soils homoionically saturated with silver thiourea in the presence of 0.015 M silver thiourea; numbers refer to soils described in Table 1.

ions in the exchange complex and the molar concentration in the equilibrium solution. Similar K_c values were found for soil organic matter, from selectivity measurements on a podzol soil of very low clay content. Corrections made on the basis of the equilibrium fluid composition and the CECs indicate an overestimate of the FES abundances by 20–30%. The statistical averages of the corrected FES values for the four soil samples are (in meq kg⁻¹):

Soil 1: 0.58 ± 0.16	Soil 3: 1.64 ± 0.12
Soil 2: 1.39 ± 0.07	Soil 4: 2.78 ± 0.18

Comparison of these numbers with CEC data in Table 1 shows that, for the soils of very low organic matter content, frayed-edge sites constitute 1% of the total cation-exchange capacity. The same is true for the heavy clay soil if allowance is made for the organic matter contribution (estimated at ~60 meq kg⁻¹) to the CEC.

Figure 2 shows Cs ion exchange isotherms at very low Cs coverages of the frayed-edge sites (0–5%). These isotherms were generated at the same AgTU background level but in the presence of 10^{-3} M K⁺ ions. Here, liquid-phase Cs concentrations range from $\sim 10^{-7}$ to $\sim 2 \times 10^{-6}$ M. The various soils show significantly different Cs sorption patterns. But if the data is expressed in terms of a Cs–K selectivity coefficient, defined as

$$K_c(\text{Cs–K}) = \frac{Z_{\text{Cs}} m_{\text{K}}}{Z_{\text{K}} m_{\text{Cs}}} \quad (2)$$

(where Z_i refers to the fraction of frayed-edge sites occupied by i) we find that as Cs concentration (and hence Z_{Cs}) $\rightarrow 0$, $K_c(\text{Cs–K})$ tends towards the same value for all four soil types (Fig. 3). Separate measurements of K_c at 'zero' Cs loading, obtained with carrier-free ¹³⁷Cs, confirmed that this limiting value of $\ln(K_c)$ is ~ 7.1 – 7.3 . The decrease in $\ln(K_c)$ with increasing Cs loading indicates the presence of two or more different types of site^{10,11}.

Evidently, this is a rather important result in that it quantitatively identifies a common Cs-binding characteristic in soils of widely different texture. Because in agricultural soils the frayed-edge sites contain K⁺ predominantly (that is, $Z_{\text{K}} \sim 1$), quantitative predictions can be made of the *in situ* K_D values of radio-caesium. It can be readily shown that, at vanishing Cs⁺ loading, equation (2) becomes

$$K_c(Z_{\text{Cs}} \rightarrow 0) = \frac{K_D m_{\text{K}}}{[\text{FES}]} \quad (3)$$

where K_D refers to the solid-liquid distribution coefficient in carrier-free conditions—the normal scenario in the case of a nuclear accident. Consequently, with knowledge of field values

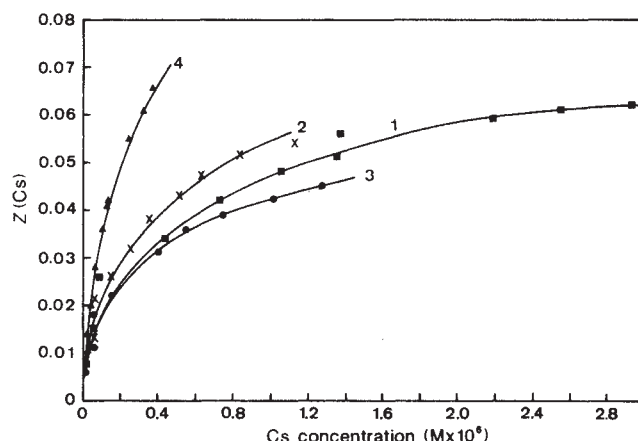


Fig. 2 Trace sorption isotherms of caesium on frayed-edge sites in the presence of 0.015 M silver thiourea and 0.001 M K⁺; Z refers to the fractional occupancy of the frayed-edge sites; numbers refer to soils described in Table 1.

of m_{K} (measurable by such methods as immiscible displacement¹²) and FES values, *in situ* predictions of K_D can be made. In future studies it would be worthwhile to correlate such data with soil-to-plant transfer patterns.

We have implied above that organic matter does not contribute to the highly specific retention of radio-caesium, the process being exclusively associated with the mineral soil component. This is corroborated by radio-caesium sorption measurements on a podzol soil with a high organic carbon content (4%), a high CEC (220 meq kg⁻¹) and a marginally low clay content (<0.1%). K_D measurements were made at zero caesium loading on the AgTU-saturated podzol soil (using carrier-free ¹³⁷Cs) in the presence of 0.015 M AgTU and 10^{-3} M KCl. The values of K_D (in l kg⁻¹), averages of 2 measurements with 2% reproducibility) are: 34 (podzol), 701 (soil 1), 1,930 (soil 2), 2,290 (soil 3) and 4,150 (soil 4). On the basis of (3), and assuming $\ln(K_c(\text{Cs–K})) = 7$, the number of FESs in the podzol soil is estimated at 0.03 meq kg⁻¹ soil, that is, $\sim 0.01\%$ of the CEC. This clearly demonstrates that soil organic matter plays no part in the specific caesium retention. The involvement of the illite

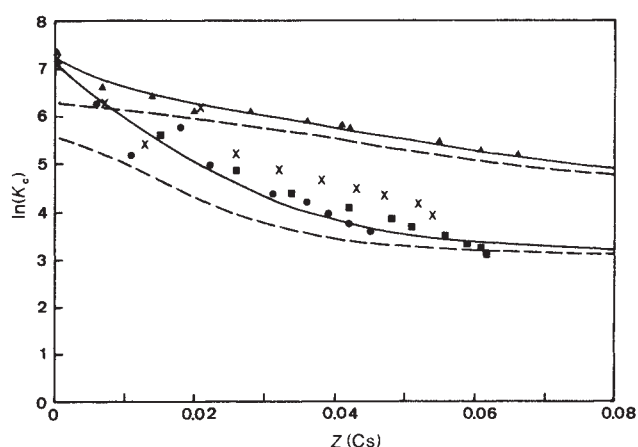


Fig. 3 Composition dependence of $\ln(K_c(\text{Cs–K}))$ for frayed-edge sites of soils; symbols correspond to those in Figs. 1 and 2. Curves (from top to bottom) refer to simulations for various combinations of fractions of site groups and characteristic selectivity coefficients. Curve 1 (full line): fraction = 0.005, $\ln(K_c) = 12.0$; fraction = 0.055, $\ln(K_c) = 9.10$; fraction = 0.940, $\ln(K_c) = 3.80$. Curve 2 (dashed line): fraction = 0.055, $\ln(K_c) = 9.10$; fraction = 0.945, $\ln(K_c) = 3.80$. Curve 3 (full line): fraction = 0.006, $\ln(K_c) = 12.0$; fraction = 0.020, $\ln(K_c) = 9.40$; fraction = 0.974, $\ln(K_c) = 2.80$. Curve 4 (dashed line): fraction = 0.020, $\ln(K_c) = 9.40$; fraction = 0.980, $\ln(K_c) = 2.80$.

component in radiocaesium interception was verified by X-ray diffraction (Cu K α radiation) on random powder specimens with particle size <50 μ m, isolated from the four soils. The spectra demonstrate the presence of micaceous and mixed-layer clay minerals. The sequence of 10-Å and mixed-layer minerals, as deduced from peak intensities, correlates with the sequence of the number of frayed-edge sites reported above.

The composition dependence of $\ln(K_c)$, shown in Fig. 3, can be described using a model with three different sites of increasing Cs selectivity: a low-selectivity site (comprising 95–97% of the FESs) characterized by $\ln(K_c)$ values $\sim$ 2.8–3.8, an intermediate-selectivity site (comprising 2–5% of the FESs) with $\ln(K_c)$ $\sim$ 9.1–9.4 and a super-selective site (comprising 0.5% of the FESs) with $\ln(K_c)$ $\sim$ 12. Simulations with and without the latter super-selective sites (full and dashed lines respectively) are shown in Fig. 3. Apart from the presence of this small proportion of super-selective sites, this model is comparable to earlier data on illite⁶ (which revealed a site with $\ln(K_c)$ = 9.4 and a site, comprising 90% of the FESs, with $\ln(K_c)$ = 3.2) providing further proof that the selective caesium retention in soils is associated with illite. The number of high affinity sites in soils ($\ln(K_c)$ = 9, 12) amounts to 0.02–0.06% of the CEC of the mineral fraction.

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Effects of protein kinase C activators on cardiac Ca²⁺ channels

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Phorbol esters have marked effects on voltage-dependent Ca²⁺ channels¹. Inhibitory² and stimulatory³ effects on cardiac Ca²⁺ channels have been attributed in both cases to activation of protein kinase C. We show that the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate stimulates dihydropyridine-sensitive ⁴⁵Ca²⁺ influx in primary cultures of neonatal rat ventricular myocytes within 5 s, but that after a 20-min pre-incubation period the phorbol ester markedly inhibits ⁴⁵Ca²⁺ influx. The sequence of stimulation followed by inhibition is confirmed in cell-attached patch clamp recordings of single Ca²⁺ channel currents. The stimulatory effect is faster at 0 mV than at -40 mV, leading to the novel conclusion that the rate of protein kinase C activation is modulated by the state of the Ca²⁺ channel.

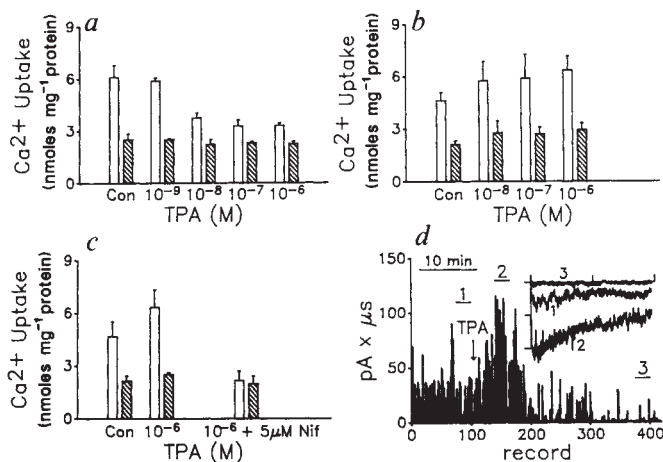


Fig. 1 Phorbol ester effects on Ca²⁺ influx and Ca²⁺ channel activity in cardiac myocytes. *a*, TPA at the indicated concentrations was added to myocytes 20 min before measuring ⁴⁵Ca²⁺ accumulation. Open bars indicate Ca²⁺ uptake in 80 mM K⁺, hatched bars indicate Ca²⁺ uptake in 5.3 mM K⁺. *b*, TPA at the indicated concentrations was added to myocytes during the 5-s measurement of ⁴⁵Ca²⁺ uptake. Bars as in *a*. *c*, Nifedipine (Nif) (5 μ M) blocks K⁺ depolarization-dependent ⁴⁵Ca²⁺ uptake in myocytes, and prevents the increase in ⁴⁵Ca²⁺ uptake following exposure to 1 μ M TPA. Error bars in all cases represent s.e.m. *n* = 3–6. Control values are labelled Con. *d*, TPA increases and then decreases Ca²⁺ currents. The integral of Na⁺ current flowing through Ca²⁺ channels in each record is shown before and after the addition of 200 nM TPA. Records lasted 390 ms and were separated by 6.27 s. The membrane was depolarized to -40 mV from a holding potential of -70 mV. In segments labelled 1, 2 and 3, 26 consecutive records were summed to produce the average currents shown in the inset. TPA was added dropwise to the bath. Inset: axes intersect at 0 pA and 0 ms. Calibrations are 200 ms and 1 pA. Filter corner frequency (*f_c*) at -3 dB attenuation was 500 Hz.

Methods. Primary cultures of neonatal rat ventricular cells were obtained by a modification of the method in ref. 19 and grown in 35-mm Falcon culture plates. Three to four-day-old cultures were transferred to a 1-ml solution (in mM): NaCl, 110; KCl, 5.3; EGTA, 0.2; HEPES, 20; glucose, 25; pH 7.4 (EGTA solution). After 5-min equilibration, this solution was replaced with 1 ml of (in mM): NaCl, 110; KCl, 5.3; CaCl₂, 1.8; MgCl₂, 1.0; HEPES, 20; glucose, 25; pH 7.4 or with a 1-ml solution having 80 mM KCl in equimolar substitution for NaCl; both solutions were supplemented with 10 μ Ci ml⁻¹ ⁴⁵Ca²⁺. After 5 s, the ⁴⁵Ca²⁺-containing solutions were aspirated and the cells washed rapidly 3 times with 1.5-ml aliquots of ice-cold EGTA. Cells were digested with 0.5% NaOH and ⁴⁵Ca²⁺ accumulation was quantitated by liquid scintillation spectroscopy. Protein concentration was determined by the method of Lowry²⁰. Procedures for formation of cell-attached patches and the acquisition and analysis of single-channel data have been described²¹. To step the patch membrane to known potentials, cells were depolarized to $\sim$ 0 mV with a bath solution containing (in mM): potassium aspartate, 140; EGTA, 10; HEPES, 10; pH 7.4. At free external divalent ion concentrations in the nM range, Ca²⁺ channels conduct Na⁺ ions at a high rate⁵. Patch pipette filling solutions consisted of (in mM): BaCl₂, 100; HEPES, 10; pH 7.4 or NaCl, 130; EDTA, 10; CaSO₄, 0.6; HEPES, 10; tetrodotoxin, 0.010; pH 7.4. Drugs were added dropwise to a static bath or pressure-ejected from a second micropipette of tip diameter $\sim$ 4 μ m. Data acquisition was halted for 6–12 s during direct addition of drugs to the bath, but not during pressure ejection. TPA and DOG stock solutions were made in dimethylsulphoxide which was present after dilution at a final concentration $\leq$ 0.1%.

We found that neonatal rat ventricular myocytes respond to an increase in extracellular K⁺ from 5.3 to 80 mM with enhanced ⁴⁵Ca²⁺ influx (Fig. 1*a*). 12-*O*-tetradecanoylphorbol-13-acetate (TPA) applied for 20 min at 10⁻⁸ to 10⁻⁶ M significantly reduced K⁺-stimulated ⁴⁵Ca²⁺ uptake (*P* < 0.05 in paired Student's *t*-test). To investigate the kinetics of the TPA effect, we exposed cardiac cells to TPA only during the 5 s when ⁴⁵Ca²⁺ influx was

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measured. Surprisingly, TPA at $\geq 10^{-8}$ M consistently increased $^{45}\text{Ca}^{2+}$ influx in both 80 mM K^{+} and 5.3 mM K^{+} (Fig. 1b); at 10^{-6} M the effect was statistically significant ($P < 0.05$, Student's paired t -test). Nifedipine blocked the increase in $^{45}\text{Ca}^{2+}$ uptake, suggesting that TPA was acting on the dihydropyridine (DHP)-sensitive high-threshold voltage-dependent Ca^{2+} channel. The diacylglycerol analogue 1,2-dioctanoyl glycerol (DOG) at 2 μM mimicked the acute effect of TPA on $^{45}\text{Ca}^{2+}$ uptake ($n = 3$), but unlike TPA increased $^{45}\text{Ca}^{2+}$ influx after 20-min exposure as well ($n = 3$). 4 α -phorbol does not stimulate protein kinase C (PKC) activity and did not show either an immediate or a delayed (after 20 min) effect ($n = 3$).

We used cell-attached patch clamp recordings for direct observation of the effects of TPA on single cardiac Ca^{2+} channel currents. We preferred cell-attached patches to whole-cell current measurements because of their greater resolution, and because whole cell currents failed to respond to TPA or DOG, probably as a result of loss into the patch pipette of intracellular components of the PKC cascade⁴. Single Ca^{2+} channel currents were conducted either by Ba^{2+} or, in the absence of divalent cations, by Na^{+} (ref. 5) and had the properties of high-threshold (L) Ca^{2+} channels⁵⁻⁸. Figure 1d shows the integral of the single Ca^{2+} channel currents conducted by Na^{+} during each step depolarization, and the entire data set of 420 records shows that the increase was followed by a reduction in a sequence similar to the sequence that occurred in the $^{45}\text{Ca}^{2+}$ uptake experiments. From the maximal number of overlapping unitary events, at least four channels must be present in this patch. The late and almost complete inhibition was not due to loss of channels from the patch because overlaps of three unitary events were seen during the late inhibitory phase.

We focused on TPA's stimulatory effect, which is mimicked by angiotensin II (ref. 3), because until recently TPA's inhibitory effects only had been described in heart³. Under control conditions we never observed a spontaneous increase in the probability of opening (p_o) of single Ca^{2+} channel currents, so the stimulatory effect of TPA after a latency of 141 s was obvious (Fig. 2a). DOG at 2 μM produced qualitatively similar effects (data not shown). The inactive 4 α -phorbol at 200 nM had no effect (Fig. 2f). Nifedipine at 2 μM blocked the TPA-induced activity (Fig. 2g). The results were similar when Ba^{2+} was the charge carrier. Analysis of data from three other patches demonstrated that TPA increased the number of openings per trace without increasing the mean open time (Fig. 2d and e). Openings occurred in bursts and the bursts were clustered. TPA produced large increases in the number of bursts per cluster. Unlike TPA-stimulated Ca^{2+} channels in *Aplysia* which are not present in control⁹, TPA-induced single-channel cardiac Ca^{2+} currents had a mean amplitude statistically indistinguishable from control values, and had similar voltage dependence. Thus the TPA-mediated increase in Ca^{2+} channel activity arises from a modification by PKC of the pre-existing DHP-sensitive Ca^{2+} channels.

The measurements of single-channel currents and $^{45}\text{Ca}^{2+}$ uptake were consistent with regard to TPA's dual stimulatory and inhibitory effects. But the onset of TPA's stimulatory effect on $^{45}\text{Ca}^{2+}$ uptake was at most 5 s, whereas TPA's stimulatory effects on p_o had latencies of 120 s or more, a result consistent with other published data^{3,4}. Two differences were the lack of control of membrane potential in the uptake experiments and the use of step depolarizations in the single-channel experiments. Damaged cells would be expected to increase the range of membrane potentials present during the uptake experiments, whereas the voltage steps we used usually held the membrane potential at -70 mV. To examine the possibility that the difference in latencies was dependent on potential TPA or DOG was applied at steady membrane potentials of either -40 or 0 mV. Transient Ca^{2+} channels are inactivated at these membrane potentials¹⁰. At both these potentials Na^{+} currents flowing through Ca^{2+} channels persisted, although as the control periods

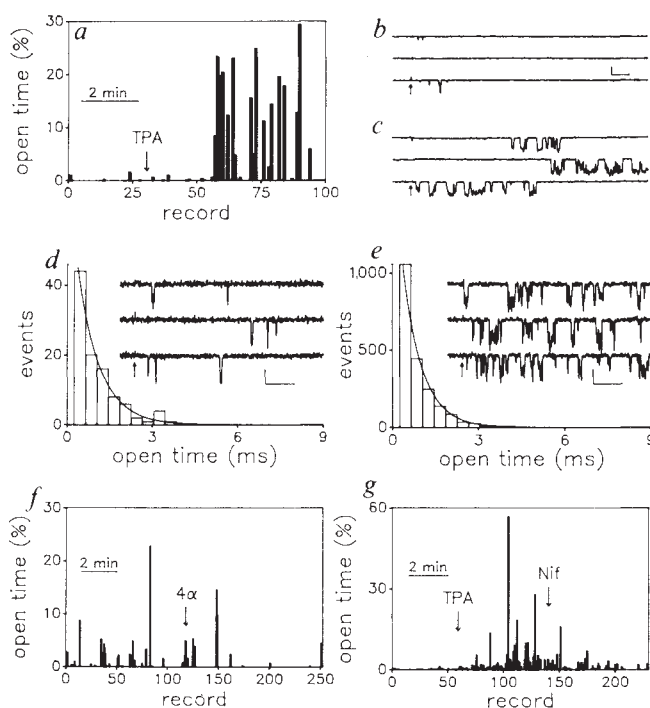


Fig. 2 TPA, but not 4 α -phorbol, activates DHP-sensitive single cardiac Ca^{2+} channels. **a**, Cell-attached patch with NaCl solution in the pipette. The patch was held at -90 mV and stepped to -30 mV for 700 ms at 5.44-s intervals. TPA was added to the bath to a final concentration of 200 nM. No overlapping events were observed in this patch and we assume that only one active channel was present. **b**, Records 1, 2 and 24 from **a** in the absence of TPA are shown. **c**, Records 56, 57 and 58 from **a** in the presence of TPA are shown. In **b** and **c**, onset of the pulse is indicated by vertical arrows. Calibrations are 50 ms and 3 pA; f_c was 300 Hz and prolonged the openings. **d** and **e**, Open-time histograms in the absence (**d**) or presence of TPA at 200 nM (**e**). NaCl solution was in the pipette which was held at -80 mV and stepped to -40 mV at 3.22-s intervals; f_c was 1.5 kHz. Open-time values of 0.78 ms (**d**; $n = 103$) and 0.67 ms (**e**; $n = 2,177$) for non-overlapping events were obtained from maximum likelihood estimation. Insets show corresponding representative records. Arrows indicate onset of the pulses. Calibrations are 20 ms and 2 pA. **f**, Cell-attached patch in the same solution conditions as **a** was held at -70 mV and stepped to -40 mV for 390 ms at 3.44 s intervals. 4 α -phorbol was added to the bath to a final concentration of 200 nM at the arrow and had no effect. A gradual decline in single Ca^{2+} channel currents was commonly observed. **g**, TPA stimulation of Ca^{2+} channels occurs when Ba^{2+} at 100 mM is the charge carrier. The patch was held at -40 mV and stepped to 10 mV at 3.44 s intervals. TPA and nifedipine were added to final concentrations of 200 nM and 2 μM respectively.

in Fig. 3 show, sojourns in a long-lived closed state occurred⁷. Latencies were defined as the time to reach the large abrupt increase in p_o , see Fig. 3a and b. At less negative membrane potentials, the latency with which TPA or DOG stimulated single Ca^{2+} channel currents was dramatically shortened (Fig. 3).

As regards to mechanism, we assume that TPA and DOG produce their stimulatory effects by activation of PKC. PKC effects on the cyclic AMP cascade¹¹, which increases the p_o of single cardiac Ca^{2+} channels^{12,13}, were ruled out because measurements of total myocyte cAMP under conditions identical to those used in the $^{45}\text{Ca}^{2+}$ uptake experiments showed no significant difference in cAMP content between control and TPA (200 nM)-treated myocytes after a 2-min incubation, in agreement with other observations^{14,15}. Also cAMP never produces inhibition of single Ca^{2+} channel currents. Therefore we conclude that PKC activation of single Ca^{2+} channel currents is

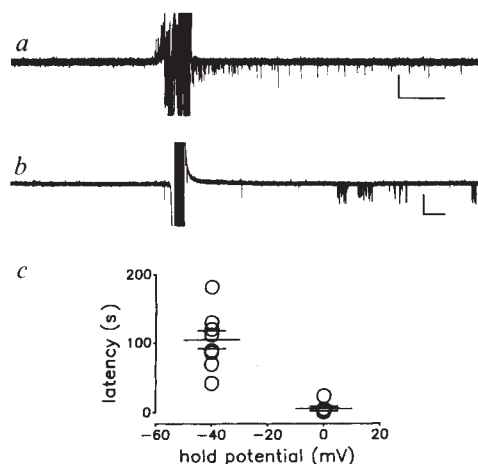


Fig. 3 PKC stimulation has a latency that is voltage dependent. Na^+ ions were conducted through Ca^{2+} channels in cell-attached patches held continuously at 0 or -40 mV. *a*, Currents in a patch held at 0 mV. DOG at $2 \mu\text{M}$ was added to the bath at the large oscillations. Calibrations are 10 s and 1 pA. *b*, Currents in another patch held at -40 mV. 50 nM TPA was added to the bath at the large oscillations. After a 69-s delay, a cluster of Ca^{2+} channel openings is seen. Calibrations in *b* are 10 s and 2 pA. Latencies were always similar to those shown, regardless of whether addition of TPA or DOG was to a static bath or by pressure ejection directly on to the cell. Records in *a* and *b* are taken from a pen recorder, and channel amplitudes are attenuated by the pen's frequency response ($f_c \approx 125$ Hz). A summary of latencies at -40 mV and 0 mV holding potentials is shown in *c*. Latencies at -40 mV and 0 mV were obtained with TPA at 50–200 nM and in three observations at 0 mV with DOG at 0.5 to $2 \mu\text{M}$. Latencies were independent of concentration over the ranges used. Mean latency at -40 mV was 104 ± 13 s ($n = 9$, s.e.m.), and at 0 mV was 6 ± 4 s ($n = 6$, s.e.m.). The latter was not distinguishable from the 5–10 s required for application of the PKC activator. Mean values were significantly different ($P = 0.001$ in Cochran and Fox t -test for unequal variances). Mean and s.e.m. are shown in *c* as wide and narrow horizontal bars respectively, and individual observations as open circles.

independent of the cAMP cascade. The PKC effects probably result from phosphorylation of the DHP receptor which is part of the Ca^{2+} channel. PKC has been shown to phosphorylate the DHP receptor purified from skeletal muscle *in vitro*, and the phosphorylated residues are different from those phosphorylated by protein kinase A (ref. 16).

One important result of our study is that TPA, an activator of PKC, produces biphasic effects on cardiac Ca^{2+} currents. The inhibitory effect requires more prolonged TPA exposure and may result from down-regulation of PKC^{14,17}. If so, this implies that some fraction of control p_0 is due to basal PKC activity. But cellular events other than C-kinase activation are likely to be involved¹⁸ as DOG did not produce inhibition. In addition, inhibition is not linked to Ca^{2+} entry, because the single-channel currents were recorded in the absence of extracellular Ca^{2+} . Our second major result is that the rate of stimulation of Ca^{2+} currents by PKC activation is clearly potential-dependent. One possibility is that the susceptibility of the Ca^{2+} channel to modulation by PKC-mediated phosphorylation specifically, or to modulation in general, depends on the state of the Ca^{2+} channel.

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Activation at M-phase of a protein kinase encoded by a starfish homologue of the cell cycle control gene *cdc2*⁺

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In both starfish and amphibian oocytes, the activity of a major protein kinase which is independent of Ca^{2+} and cyclic nucleotides increases dramatically at meiotic and mitotic nuclear divisions^{1–6}. The *in vivo* substrates of this kinase are unknown, but phosphorylation of H1 histone can be used as an *in vitro* assay. We have purified this kinase from starfish oocytes. The major band in the most highly purified preparation contained a polypeptide of relative molecular mass (M_r) 34,000 (34K). This is the same size as the protein kinase encoded by *cdc2*⁺, which regulates entry into mitosis in fission yeast^{7–11} and is a component of MPF purified from *Xenopus*¹². Here, we show that antibodies against p34 recognize the starfish 34K protein and propose that entry into meiotic and mitotic nuclear divisions involves activation of the protein kinase encoded by a homologue of *cdc2*⁺. Given the wide occurrence of *cdc2*⁺ homologues from budding yeast to *Xenopus* and human cells^{9,11,12}, this activation may act as a common mechanism controlling entry into mitosis in eukaryotic cells.

When starfish oocytes arrested at the first meiotic prophase are released by hormonal stimulation, they undergo two nuclear divisions^{13,14}. A protein kinase that is independent of Ca^{2+} and cyclic nucleotides, phosphorylates serines and threonines, and uses H1-histone as an *in vitro* substrate, increases by about 30-fold just before first meiotic metaphase in dilute extracts prepared from oocytes, and drops to a low level at ana-telophase. A similar peak in activity is seen during the second meiotic nuclear division and during the first mitotic cell cycles in the early embryo^{4,14}. More than 90% of this M-phase protein kinase activity elutes as a single peak on a DEAE ion-exchange column (Fig. 1 and ref. 15). During subsequent column chromatography on hydroxyapatite, phosphocellulose, TSKG 3000 and Mono Q, a single peak of activity is found at each step (Fig. 1). One major protein of M_r 34K and two minor proteins of M_r 42K and 200K are observed at the peak of activity on the final column (Fig. 2). The two larger proteins are unlikely to have the protein

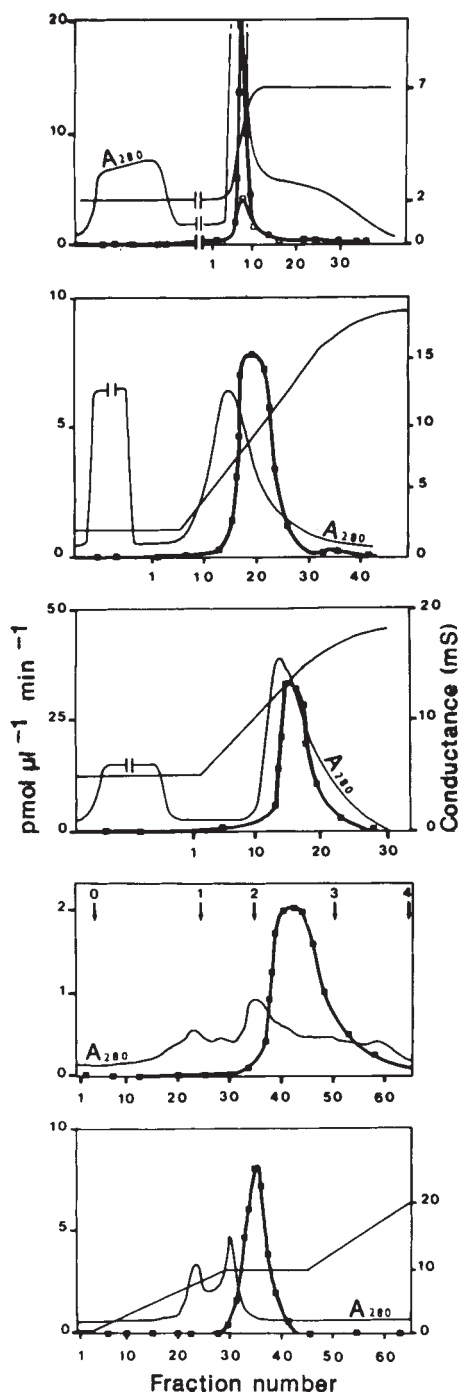


Fig. 1 Purification of the M-phase protein kinase from starfish oocytes. From top to bottom: the successive steps of kinase purification by column chromatography on DEAE-cellulose (top), hydroxyapatite, phosphocellulose, TSKG 3,000 and Mono Q (bottom). DEAE-cellulose: homogenates were prepared from oocytes either at first meiotic metaphase (■) or arrested at first meiotic prophase (□), in a buffer containing 80 mM glycerophosphate, 15 mM EGTA, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT) at pH 7.8. The supernatant (40 ml: 700 mg of proteins) was diluted to 2 mS and applied to a 145-ml column of DEAE-cellulose equilibrated with 5 mM glycerophosphate, 1.5 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT (buffer A) containing 35 mM NaCl at pH 7.0. The proteins were eluted in one step at 7 millisiemens (7 ml fractions were collected). Only the H1-histone kinase activity originating from M-phase oocytes was further purified. Hydroxyapatite: the active fractions were pooled, diluted to 2 millisiemens and applied to a 20-ml hydroxyapatite column equilibrated with a 20 mM phosphate buffer at pH 7.0 containing 1 mM DTT. After washing the column (2 bed volumes) a 100-ml linear gradient (from 20 to 500 mM phosphate buffer) was applied, and 1.7 ml fractions collected. Phosphocellulose: the active fractions, eluting from the hydroxyapatite column at about 10 mS were pooled, diluted to 5 mS and applied to a P11 phosphocellulose column (2 ml) equilibrated with buffer A containing 100 mM NaCl, which was developed with a 50–500 mM NaCl gradient (fractions of 0.7 ml were collected). TSKG 3,000: the peak of histone kinase activity eluting at about 13 mS was concentrated by ultrafiltration and applied to a 21.5 × 600 mm TSKG 3,000 HPLC column equilibrated with buffer A containing 100 mM NaCl and fractions (3 ml) were collected. Arrows point to the elution volumes of 0, bovine thyroglobulin (M_r , 670,000); 1, bovine globulin (M_r , 150,000); 2, chicken ovalbumin (M_r , 44,000); 3, horse myoglobin (M_r , 17,800); 4, vitamin B_{12} (M_r , 1,350). Mono Q: the H1-histone phosphorylating activity eluted between the markers of M_r , 17,800 and 44,000 from the TSKG 3,000 column and this peak was diluted twice and applied to a Mono Q column of a Pharmacia FPLC system equilibrated with a buffer containing 25 mM Tris, 1 mM DTT and 1 mM EGTA at pH 7.8. After washing the column, proteins were eluted (0.3 ml fractions) with a discontinuous linear gradient of NaCl (0–0.6 M) made in the same buffer. In all experiments, assays for kinase activity contained the following in a final volume of 40 μ l: 1 mg ml^{-1} of histone H1 (Sigma type IIIS); 200 μ M [32 P] ATP (100 c.p.m. $pmol^{-1}$); 10 mM $MgCl_2$; 20 mM HEPES pH 7.4. Assays were terminated by spotting 35 μ l aliquot on to pieces of P81 phosphocellulose paper, which were washed and counted by scintillation¹⁹.

kinase activity, because they are present at higher levels in other fractions which have reduced protein kinase activities (Fig. 2, lane A). Protein kinase activity coincided with the 34K protein both on the final column and during the two previous steps of purification. Therefore the 34K protein is the best candidate for the M-phase protein kinase.

The cell-cycle control gene *cdc2⁺* of the fission yeast and its homologues *cdc28* in budding yeast and *CDC2* in human cells, encode protein kinases of the same M_r as the starfish M-phase protein kinase^{7–11}. All three homologues contain an identical 16-amino-acid region EGVSTAIRESLLKE which differs from all other sequenced protein kinases⁹. Affinity-purified polyclonal serum raised against this peptide detects a single 34K band in Western blots of yeast and mammalian cell extracts⁹. We have found that this serum cross-reacts with the purified starfish M-phase specific 34K protein kinase (Fig. 2), and that

immunoprecipitation using the serum removes all H1 kinase activity from the purified material and about half the activity from crude extracts. The serum also recognizes a single 34K band in crude starfish oocyte extracts and immunoreactivity co-purifies with the H1 kinase during the course of purification. These results indicate that the starfish M-phase protein kinase is encoded by a starfish homologue of the *cdc2⁺* gene.

Protein synthesis is required for the M-phase protein kinase activity to reappear after its drop at ana-telophase of each cell cycle^{4,14}. Nonetheless, a Western blot using the anti-peptide serum did not show any decrease in the level of the protein at the time of first meiotic cleavage, even when the second meiotic nuclear division was suppressed using the protein synthesis inhibitor emetine (Fig. 3).

The low level of H1-histone kinase activity observed in oocytes arrested at first meiotic prophase co-purifies with the M-phase

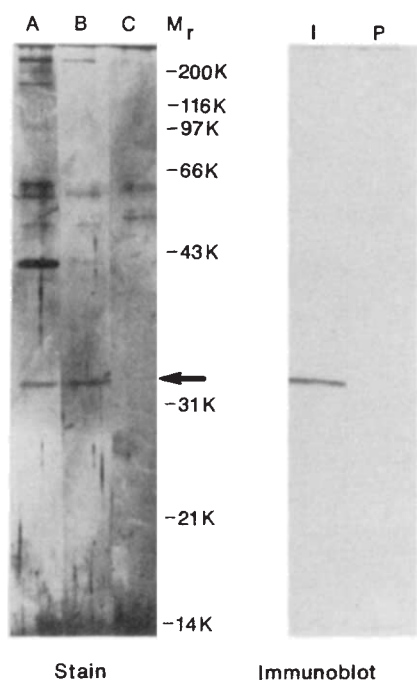


Fig. 2 Polyacrylamide gel analysis and immunoblotting of the M-phase H1-histone kinase after purification through the Mono Q-FPLC step. Left, samples of the preparation taken at the mid-height of the peak of H1-histone kinase activity (lane A), at the highest point (lane B), and of sample buffer without proteins (lane C) were electrophoresed through a 12.5% SDS-polyacrylamide gel and silver stained. M_r markers are myosin (M_r 2,000,000), β -galactosidase (M_r 116,000), phosphorylase b (M_r 97,000), serum albumin (M_r 66,000), ovalbumin (M_r 43,000), carbonic anhydrase (M_r 31,000), soybean trypsin inhibitor (M_r 21,000) and lysozyme (M_r 14,000). Two bands showing electrophoretic mobilities corresponding to M_r of 56,000 and 64,000 were present in all three lanes and correspond to artefacts due to the reaction of reducing agents with components of sample buffer^{20,21}. Right, Western blot analysis of the most purified fraction. I: a polyclonal serum raised against the peptide EGVPTSTAIPELLKE was used. P: the corresponding pre-immune serum was used.

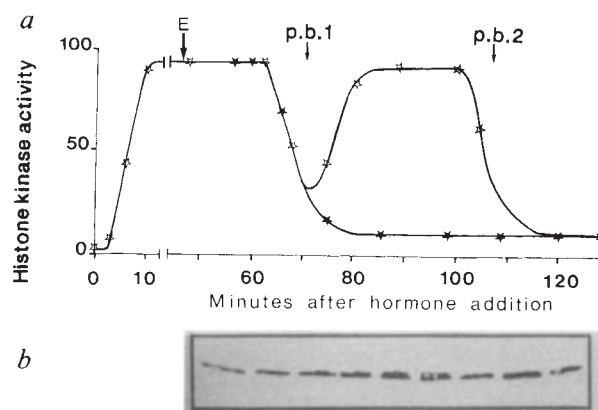


Fig. 3 *a*, Changes of the Ca^{2+} and cyclic nucleotide-independent H1-histone kinase activity during meiotic maturation induced by addition of the hormone 1-methyl adenine at time 0. Activity seen in control oocytes ($\star$) and oocytes to which 0.1 mM of the protein synthesis inhibitor emetine was added at time shown by E ($\star$). Control oocytes emitted the first and second polar bodies corresponding to the completion of each meiotic nuclear division at p.b.1 and p.b.2, while emetine-treated oocytes emitted only the first polar body simultaneously with control oocytes. Protein kinase assays were performed as follows: At the indicated times, groups of 10 oocytes (volume of each oocyte: 2 nl) were rapidly washed in an excess of buffer containing 50 mM β -glycerophosphate, 15 mM EGTA, 10 mM MgCl_2 and 0.7 mM DTT at pH 7.3; then the oocytes were taken in 5 μl of buffer and frozen in liquid nitrogen. Immediately after thawing, which disrupted the oocyte, an identical volume of a mixture containing 100 μM [γ - ^{32}P]ATP (1,200 c.p.m. pmol^{-1}), 10 mM MgCl_2 and 2 mg ml^{-1} lysine-rich histones (type II S from Sigma) was added. After 10 min of incubation at 21 $^\circ\text{C}$, the reaction was stopped by addition of 30 μl of a mixture containing 30% urea, 5% SDS, 8% DTT (w/v), in 0.5 M Tris-HCl pH 6.8, and the proteins were separated by SDS-polyacrylamide gel electrophoresis. The parts of the gels containing H1-histone were cut out and counted by liquid scintillation. *b*, Western blot analysis showing the levels of the 34 K protein from 25 to 175 min after hormone addition in oocytes treated with emetine at 40 min. Equal amounts of protein were loaded on the gel from samples prepared from the same population of oocytes as used for measuring changes of protein kinase activity, at the following times (from left to right): 25, 50, 75, 85, 100, 125, 135, 150 and 175 min. Similar results were also seen with the control oocytes (data not shown).

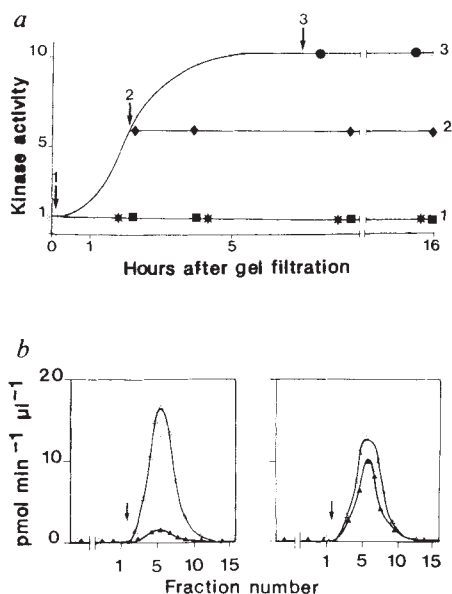


Fig. 4 *a*, H1-histone kinase activation in gel-filtered extracts prepared from prophase-arrested oocytes. A homogenate (8 mg protein ml^{-1}) was prepared as described in the Fig. 1 legend. Aliquots were taken after various incubation times at 22 $^\circ\text{C}$ and the Ca^{2+} and cyclic nucleotide-independent H1-histone kinase activity when assayed as described in Fig. 1, with activities normalized to initial values equal to 1. Control extracts ($\blacksquare$) and extracts from which the small metabolites including ATP had been eliminated from the high speed supernatant by rapid gel-filtration at 4 $^\circ\text{C}$ ($\circ$). 1 mM ATP was added back to the gel-filtered extract immediately after filtration at arrow 1 ($\star$), after 2 h at arrow 2 ($\diamond$), and after 7 h at arrow 3 ($\bullet$). *b*, DEAE-cellulose column chromatography of the Ca^{2+} and cyclic nucleotide-independent H1-histone kinase activities in extracts prepared from non-hormone-stimulated oocytes arrested at first meiotic prophase ($\blacktriangle$) or from hormone-stimulated oocytes at first meiotic metaphase ($\triangle$). Left, high speed supernatants (17 mg protein ml^{-1} in both cases) were applied to the column immediately after centrifugation. Right, low M_r metabolites (including ATP) were eliminated by rapid gel filtration from the high speed supernatants which were subsequently left for 8 h at 22 $^\circ\text{C}$ before application to the column. Arrows indicate the times at which the ionic strength of the buffer was raised to 7 mS with NaCl.

specific protein kinase on the DEAE column (Fig. 4, lower section) and subsequent stages of purification, although its final specific activity is 10–15 times smaller. The activation of this protein kinase on entry into nuclear division can be mimicked *in vitro*. Extracts from immature oocytes contain the unactivated level of the protein kinase but if low relative molecular mass components are first eliminated by gel filtration using a G25-Sephadex column, the activity increases about 10-fold after a lag of 30 min (Fig. 4, top). The *in vitro* activated kinase co-chromatographed with the M-phase specific kinase on the DEAE column (Fig. 4, bottom) and at subsequent stages of purification (data not shown), suggesting that the *in vivo* and *in vitro* activated enzymes are similar. The *in vitro* activation can be inhibited by addition of 0.3 mM ATP to the gel-filtered extract, but such an addition does not depress protein kinase activity once it has been activated (Figure 4, top). Neither γ -thiophosphate nucleoside triphosphates, nor non-hydrolysable ATP analogues like AMP-PNP or AMP-PCP can substitute for ATP in inhibiting protein kinase activation. These results indicate there is a progressive inactivation of an inhibitory component which requires phosphorylation to maintain its inhibitory effect. A putative protein kinase, *weel*⁺ exerts negative control over the *cdc2*⁺ gene function at mitosis in fission yeast and it has been proposed that a second putative protein kinase *niml*⁺ inhibits *weel*⁺ activity^{16,17}.

It has recently been shown that p34^{cdc2} is a component of *Xenopus* maturation promoting factor (MPF)¹² which can induce meiotic nuclear division in *Xenopus* oocytes, and results with *Physarum polypephalum* have also implicated a H1-histone

kinase in the initiation of mitosis¹⁸. Our data indicate that purified starfish M-phase H1-histone kinase contains p34^{cdc2} and therefore we propose that the activation of the *cdc2*⁺-encoded protein kinase acts as a common mechanism controlling entry into mitosis in eukaryotic cells.

Note added in proof: Other workers have now also provided evidence that p34^{cdc2} protein kinase peaks in activity at M-phase²² and is a component of *Xenopus* MPF²³.

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Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT_{1B} receptors coupled to a G_i-protein

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Growth factors can be divided into two classes which act through distinct signal transduction pathways¹. One class including epidermal growth factor, platelet derived growth factor and fibroblast growth factor activates receptor tyrosine kinases^{2,3}, and the second class, including thrombin^{4,5}, bombesin^{6,7}, bradykinin⁷ and vasopressin^{7,8} activates a phosphoinositide-specific phospholipase C through GTP-binding proteins⁹ which can be inactivated by pertussis toxin¹⁰. In Chinese hamster lung fibroblasts, thrombin-induced mitogenicity seems to correlate well with phospholipase C activation and both events are sensitive to pertussis toxin^{1,5,11}. Thrombin^{12,13}, like the other mitogens in this class^{14–16}, simultaneously inhibits adenylate cyclase. This involves an inhibitory G protein (G_i), a well established pertussis toxin substrate^{17,18}. The relative contributions of the two signalling pathways to mitogenicity has not been evaluated so far. We report here that the neurotransmitter serotonin (5-hydroxytryptamine), a contracting agent¹⁹ and mitogen for smooth muscle cells²⁰, activates phospholipase C, inhibits adenylate cyclase and stimulates DNA synthesis in fibroblasts. These events are sensitive to pertussis toxin. We show that the mitogenicity of 5-hydroxytryptamine can be uncoupled from phospholipase C activation that is mediated by 5-HT₂ receptors, but correlates perfectly with inhibition of adenylate cyclase through 5-HT_{1B} receptors. We propose that inhibition of adenylate cyclase or activation of an undefined effector system by G_i is important in 5-hydroxytryptamine induced DNA synthesis and contributes to the strong mitogenicity of the other members of this family of growth factors.

The effect of 5-hydroxytryptamine (5-HT) on reinitiation of DNA synthesis in quiescent CHL fibroblasts (CCL39 cell line) is shown in Fig. 1a. On its own 5-HT has no appreciable effect but it greatly potentiates the mitogenicity of FGF (fibroblast growth factor). Epidermal growth factor and insulin (data not shown) is also amplified by 5-HT; the maximal response however, remains well below that obtained with FGF. 5-HT does not act in synergy with thrombin, suggesting that the two factors use a common signalling pathway (data not shown).

5-HT activates the release of inositol phosphates (IPs) and inhibits stimulated adenylate cyclase (AC) (Fig. 1b and c). Both dose-response curves correlate well with mitogenicity. Inhibition of AC could be completely abolished with pertussis toxin (PT), whereas IP release was only reduced. We have used the highly developed pharmacology of 5-HT receptors^{21,22} to identify those involved in mediating phospholipase (PLC) activation, AC inhibition and mitogenicity in CCL39 cells (Fig. 2). The 5-HT₂ receptor antagonists ketanserin²³ and ritanserin selectively blocked IP release (Fig. 2b), but had no effect on the inhibition of AC (Fig. 2c) or on mitogenicity (Fig. 2a). Cyclic AMP formation, therefore is not due to stimulation of the cAMP phosphodiesterase by Ca²⁺ released as a result of PLC activation²⁴. The 5-HT_{1B}-receptor antagonist SDZ 21-009 (ref. 25) reversed inhibition of AC (Fig. 2c) and surprisingly completely blocked 5-HT-induced mitogenicity (Fig. 2a), leaving the FGF response intact. This is a specific effect: concentrations of antagonist below 10⁻⁵ M do not interfere with mitogenicity, IP production or AC inhibition by thrombin and other growth factors tested. At high concentrations AC was stimulated (Fig. 2c). This effect was dependent on the presence of 5-HT, could be abolished with ketanserin and is probably a consequence of the activation of protein kinase C by 5-HT₂-receptors (unpublished observations). Similar results have recently been described for thrombin¹³. There was only partial inhibition of IP release (Fig. 2b) and this was not in addition to the inhibition produced by PT (data not shown), possibly reflecting a role for cAMP as a negative modulator of PLC activity¹³. The antagonist of the 5-HT_{1A} and 5HT₂-receptors, Spiperone^{21–23}, blocked IP release at concentrations in the nanomolar range (data not shown),

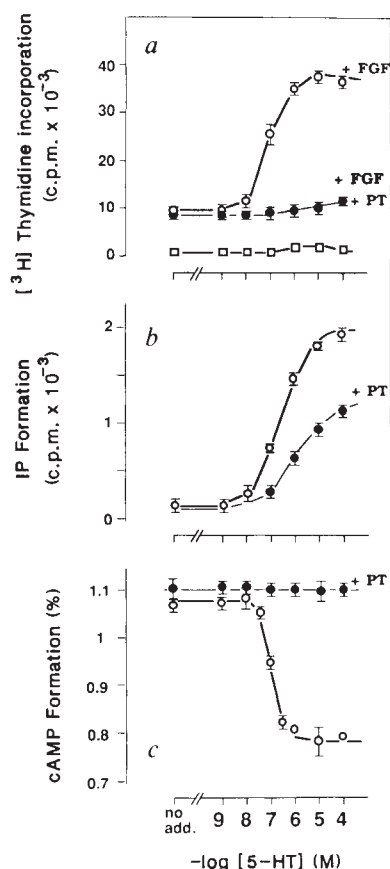


Fig. 1 5-HT potentiates the mitogenicity of FGF (a), stimulates inositol phosphate formation (b) and inhibits cAMP formation induced by cholera toxin (c). Dose-response curves for 5-HT. Error bars indicate standard error of the mean of duplicate or triplicate determinations. a, $\square$: 5-HT added alone; $\circ$, $\bullet$: in the presence of FGF (25 ng ml^{-1}); $\bullet$: pertussis toxin (20 ng ml^{-1}) was added 5 hours before the growth factors. b, $\circ$: 5-HT added alone; $\bullet$: pertussis toxin was added 5 hours before the beginning of the experiment. c, $\square$: 5-HT added alone; $\bullet$: pertussis toxin was added 5 hours before the beginning of the experiment.

Methods. Confluent cultures of CHL fibroblasts (CCL39 line) cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum were rendered quiescent by complete deprivation of serum for 24 hours. Then a 1:1 mixture of DMEM/Ham's F12 medium containing $[^3\text{H}]$ thymidine ($1 \mu\text{Ci ml}^{-1}$) and the growth factors indicated were added. After another 24 h cell monolayers were fixed with 5% trichloroacetic (TCA) acid and washed. Acid-insoluble material was collected with 0.1 M NaOH and counted by liquid scintillation spectrometry. Recombinant basic FGF was a gift from Prof. D. Gospodarowicz. 5-HT was from SIGMA, and PT was from LIST Biological Lab. b, IP formation was measured following a modified protocol¹⁶ adapted from Berridge *et al.*³⁷. Cells were rendered quiescent as described above in serum-free DMEM supplemented with $[^3\text{H}]$ adenine ($2 \mu\text{Ci ml}^{-1}$). Conversion of $[^3\text{H}]$ ATP to cyclic $[^3\text{H}]$ AMP was measured in HEPES-buffered DMEM. AC was stimulated with cholera toxin (100 ng ml^{-1}) and 20 min later the agonists were added. After another 10 min isobutyl-methylxanthine [IBMX] (1 mM) was added to inhibit the phosphodiesterase and 10 minutes later the cells were extracted with ice-cold 5% TCA. $[^3\text{H}]$ ATP and $[^3\text{H}]$ cAMP were separated by sequential chromatography on Dowex and alumina columns¹³. The ordinate shows the $[^3\text{H}]$ cAMP/ $[^3\text{H}]$ ATP ratio (%).

without affecting inhibition of AC or mitogenicity. This eliminates activation of PLC by the 5-HT_{1C} receptor as a possible mechanism²². An antagonist directed against 5-HT_3 receptors, SDZ 205-930 (ref. 21), had no effect.

To confirm that the mitogenic effect of 5-HT is mediated through 5-HT_{1B} receptors, we used the 5-HT_{1B} agonist 1-(3-

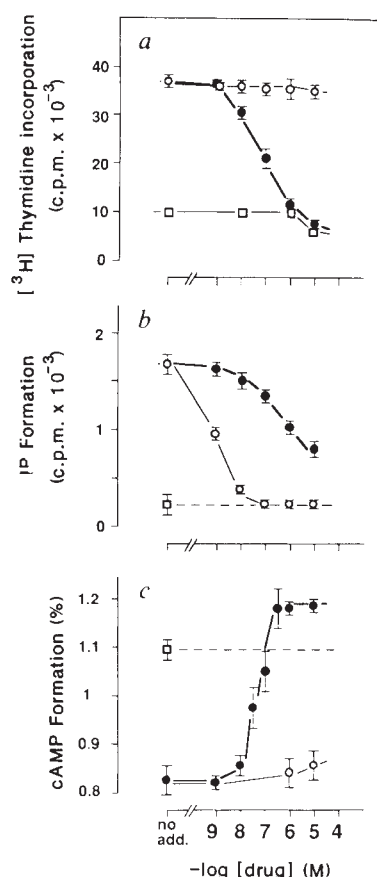


Fig. 2 Effect of the 5-HT antagonists ketanserin ($\circ$) and SDZ 21-009 ($\bullet$) on reinitiation of DNA synthesis (a), inositol phosphate formation (b) and inhibition of adenylate cyclase (c) by 5-HT (10^{-6} M). Dose response curves for the drugs. The drugs were added 20 min before 5-HT. Stock solutions of drugs (1 mM) were prepared freshly in 0.01 M HCl (ketanserin) or water (SDZ 21-009). The experiments were performed as described in Fig. 1 legend. a, $\square$: FGF without 5-HT (drug added is SDZ 21-009); $\circ$, $\bullet$: in the presence of FGF and 5-HT. b, $\square$: 20 mM Li^+ only; $\circ$, $\bullet$: in the presence of 5-HT. c, $\square$: cholera toxin + IBMX alone; $\circ$, $\bullet$: in the presence of 5-HT.

(trifluoromethyl)phenyl)piperazine (TFMPP)²⁶ in an attempt to mimic 5-HT action. As shown in Fig. 3, it inhibits AC and acts synergistically with FGF to reinitiate DNA synthesis. Both effects were completely inhibited by PT and SDZ 21-009 (data not shown) and there was no activation of IP release. Similar data were obtained using a second 5-HT_{1B} selective agonist, RU 24969 (ref. 27). Agonists of 5-HT_{1A} and 5-HT_3 receptors (8-hydroxy-2-(di-n-propylamino) tetraline (8-OH-DPAT) and 2-methyl-5-HT had no effect.

These results establish that: (1) inhibition of AC and activation of PLC by 5-HT are mediated by two different receptors in CHL cells (5-HT_{1B} and 5-HT_2 respectively), (2) the mitogenic effect of 5-HT is transmitted by 5-HT_{1B} receptors negatively coupled to AC and (3) even though PLC is activated by 5-HT, this level of activation is unimportant for the observed effect on reinitiation of DNA synthesis. Therefore, it is the activation of the inhibitory G protein of the AC system that is the step in the reinitiation of DNA synthesis by 5-HT which is sensitive to PT. Although it has been known for several years that the regulatory α -subunit of G_i is a PT substrate^{17,18}, it was thought that the G protein involved in reinitiation of DNA synthesis was distinct from that inhibiting AC. This was because cAMP exerts different effects on growth control in different cell systems^{28,29} and also because several distinct PT-sensitive G proteins have been discovered that may well be linked to effectors other than AC^{30,31}. The putative G protein controlling PLC activity (G_p) seemed a better candidate^{1,5,11,32}. Our results indicate the necessity for a new assessment of the relative roles of AC and PLC in growth

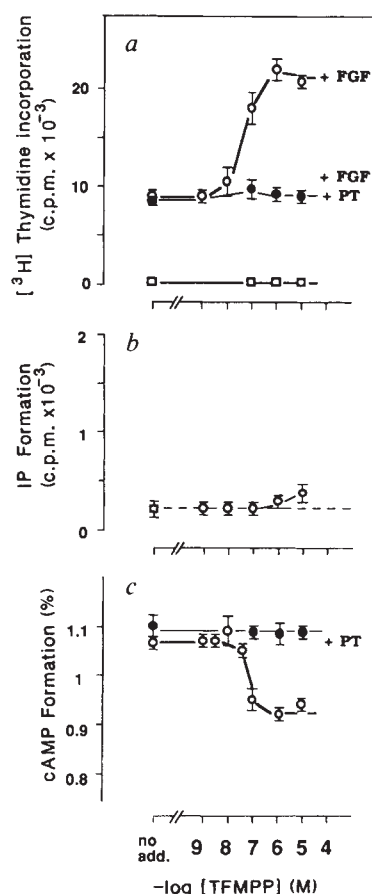


Fig. 3 Effect of the 5-HT_{1B} agonist TFMPP on reinitiation of DNA synthesis (a), inositol phosphate formation (b) and inhibition of adenylate cyclase (c). Experiments described in Fig. 1 legend. Stock solutions of TFMPP (1 mM) were prepared freshly in water. TFMPP was obtained from RBI Research Biochemicals. a, □, TFMPP added alone; ○, ●, in the presence of FGF; ●, pertussis toxin was added 5 hours before the growth factors. b □, 20 mM Li⁺ only; ○, in the presence of TFMPP; c, ○, TFMPP added alone; ●, pertussis toxin was added 5 hours before the beginning of the experiment.

control. PLC is only weakly activated by 5-HT compared to thrombin,⁵ the activation corresponding to that produced by thrombin concentrations that are not mitogenic but can maximally inhibit stimulated AC¹³ and produce the same type of synergy with FGF in reinitiation of DNA synthesis¹¹. At higher concentrations however, thrombin strongly activates PLC and becomes a potent mitogen on its own. Therefore, PLC activation may be important in mitogenicity, probably cooperating with the 'G_i pathway'.

Among the growth-promoting agents which are thought to activate PLC and simultaneously inhibit AC are thrombin, bombesin, vasopressin, bradykinin and phosphatidic acid^{4-8,11-16}. It has already been shown that the mitogenicity of bombesin can be inhibited with PT^{32,33}, without affecting PLC activation³³. Therefore, extending the conclusions drawn from our results with 5-HT, we propose that activation of G_i is an important transducing mechanism in the action of this emerging class of mitogens which includes neurotransmitters, neurohormones and vasoactive peptides. In these cases, it remains to be established whether different receptors mediate PLC stimulation and AC inhibition, or whether a dual activation process can proceed through a single receptor. The results we present here as well as data on adrenergic³⁴ and muscarinic³⁵ receptors, support the first hypothesis.

We have referred to G_i as the functional entity inhibiting AC, but at least three different forms of α_i-subunits have been described (α_{i-1} (ref. 30) and two of them, α_{i1} and α_{i2}, have

been identified in CHL cells³¹. Future experiments will show how these different G_i proteins are involved in the regulation of AC and PLC activity and possibly other, so far undefined, effector systems that could be important in the control of cell proliferation.

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Transfer of a functional human immune system to mice with severe combined immunodeficiency

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The pressing need for a better experimental system for AIDS research has brought into sharp focus the shortcomings of available animal models and the practical and ethical limitations of studies of immune responses and viral pathogenesis in humans^{1,2}. Current

Table 1 Summary of human PBL→SCID transfers

Number of PBL transferred	Donor EBV status	Route	SCID recipients analysed	Outcome
$(10-90) \times 10^6$	positive	i.v.	10	All recipients alive six months post-transfer, no human immunoglobulin production, few human lymphoid cells detected, no human tumours.
$(50-90) \times 10^6$	positive	i.p.	38	All recipients produce human immunoglobulin within 1 week of reconstitution, survival of human T, B and M ϕ documented, produce human antibody response to tetanus toxoid, develop EBV ⁺ B-cell lymphomas or lymphoproliferative disease at 8-16 weeks post-transfer.
10×10^6	positive	i.p.	22	All recipients produce human immunoglobulin within 2-3 weeks of reconstitution, survival of human T, B and M ϕ documented, antibody response to tetanus toxoid at 20 weeks post-transfer, all recipients alive at 6-26 weeks post-transfer, no gross evidence of tumours.
50×10^6	negative*	i.p.	3	All recipients produce human immunoglobulin within 1 week of reconstitution, documentation of human T, B and M ϕ survival, positive T-cell proliferative response to tetanus toxoid <i>in vitro</i> , no gross or microscopic evidence of tumours at 14 weeks post-transfer.

* An additional 10 SCID recipients were injected with PBL from an EBV-seronegative donor. These recipients developed B lymphomas which hybridized with an EBV-specific DNA probe. This donor is presumed to be EBV-positive. i.v., intravenous; i.p., intraperitoneal.

studies of the human immune responses are limited to relatively restrictive *in vivo* experiments and several *in vitro* systems that, although useful, allow only short-term studies and support responses to a few antigens². Neither model is particularly amenable to studies of the pathogenesis of diseases of the immune system. We report here that injection of human peripheral blood leukocytes (PBL) can result in the stable long-term reconstitution of a functional human immune system in mice with severe combined immunodeficiency (SCID)⁴. Human PBL transplanted to SCID mice increase in number and survive for at least six months; reconstituted mice show spontaneous secretion of human immunoglobulin and a specific human antibody response is induced following immunization with tetanus toxoid. All of the major cell populations present in PBL are found in the lymphoid tissue and blood of SCID recipients, although the relative proportions of B cells, T-cell subsets and monocytes/macrophages in long-term recipients differ from those found in normal PBL and, in mice transplanted with 50×10^6 or more PBL from Epstein-Barr virus (EBV)-seropositive donors, EBV-positive B-cell lymphomas often develop. Our results suggest that xenogeneic transplantation of human lymphoid cells into SCID mice may provide a useful model for the study of normal human immune function, the response of the immune system to pathogenic agents and early events in lymphomagenesis.

SCID mice, because of their immunodeficiency, are permissive for the growth of transformed human cell lines^{5,6}, and we therefore decided to investigate their ability to support the survival of normal, mature human lymphoid populations. (Similar investigations, using human fetal liver stem cells as the source of lymphoid cells, have also been conducted elsewhere; M. McCune and I. Weissman, personal communication.) The major problem we anticipated from transferring mature human lymphoid cells to SCID mice was the development of a lethal human graft versus mouse host (GVH) disease, so our first experiments involved transferring varying numbers of PBL by different routes into SCID mice to define conditions that minimized the anticipated GVH reaction. Table 1 summarizes the results of these experiments, from which several conclusions can be drawn: (1) xenogeneic GVH disease was not a significant problem in any recipient, although mild and transient GVH reactions may contribute to spontaneous B-cell activation. (2) Survival of human lymphoid cells depended upon intraperitoneal injection of PBL; intravenous injection was ineffective. (3) Both the EBV status of the PBL donor and the number of PBL transferred were critical. Successful establishment and maintenance of functional human lymphoid cells in SCID recipients was achieved with low numbers (10×10^6) of PBL from an EBV-positive donor or higher numbers (50×10^6)

of PBL from an EBV-negative donor. In contrast, transfer of 50×10^6 or more PBL from an EBV-positive donor resulted in both a rapid reconstitution of human immune function and development of human lymphoid tumours by 8-16 weeks after cell transfer in most mice. The tumours that were analysed by histological and immunological criteria were human B-cell lymphomas. DNA from these tumours hybridized with probes for EBV as well as human *Alu* sequences, and serum from tumour-bearing mice showed monoclonal or oligoclonal immunoglobulin patterns. A more detailed description of lymphomagenesis in these mice will be reported elsewhere.

Human immunoglobulin levels in SCID sera were measured weekly to follow B-cell survival and function. Figure 1 illustrates the outcome in three groups of SCID mice, each injected with PBL from a different donor. There was a rapid increase in spontaneous human immunoglobulin secretion in recipients with 50×10^6 PBL regardless of the EBV status of the donor. The cause of this immunoglobulin production is not understood at present, but these results indicate that it is not necessarily related to EBV transformation of B cells⁷ and may not be abnormal because transfer of normal mouse lymphoid cells to SCID mice results in similar secretion of mouse immunoglobulin (unpublished observations). Transfer of smaller numbers of PBL resulted in a slower increase in human immunoglobulin levels. SCID recipients that survived long-term (currently 16-26 weeks) showed stable levels of human immunoglobulin ranging from 0.1 to 1.0 mg ml⁻¹ (normal range of human serum immunoglobulin is 7-24 mg ml⁻¹), whereas recipients of higher numbers of PBL from EBV-seropositive donors continued to increase immunoglobulin production up to 5 mg ml⁻¹.

The demonstration that a functionally intact human immune system can survive in SCID recipients is of fundamental importance for the potential utility of the model. We immunized PBL-reconstituted SCID mice with tetanus toxoid, a protein antigen to which the PBL donors were known to be immune, and measured the antibody response 1-3 weeks later. Figure 2 shows the results of mice immunized within three weeks following transfer of 50×10^6 PBL. Eight of 10 animals produced detectable antibody to tetanus toxoid, with those which responded earliest generally displaying the highest antibody titre. The amount of antibody found in responding mice ranges from 1 to 10% of the amount found in immunized humans⁸. Two SCID recipients of 10×10^6 PBL were immunized at 20 weeks after PBL reconstitution, and both of these mice produced antibody to tetanus toxoid at about 1% of the level of normal humans (see Fig. 2 legend). This experiment suggests that functional human antigen-presenting cells, helper T cells and B cells persist for at least 20 weeks in SCID recipients, although a role

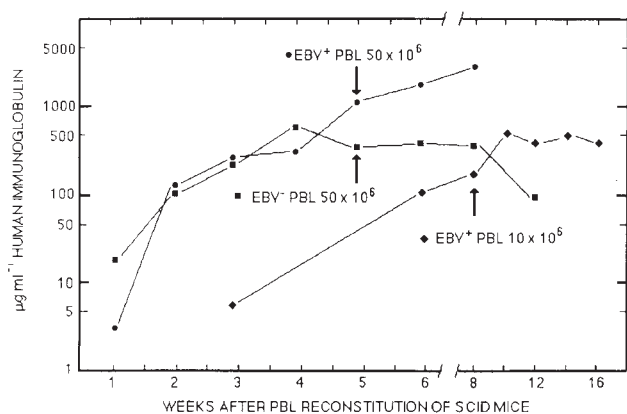


Fig. 1 Human immunoglobulin levels in the serum of SCID mice at intervals of time following intraperitoneal (i.p.) injections of human PBL.

Methods. PBL from EBV-seropositive and seronegative donors were prepared by Ficoll-Hypaque separation. Sterile PBL suspensions were injected i.p. into 8–10-week-old C.B-17 SCID mice (initially supplied by Drs Mel Bosma and Ken Dorschkind) previously shown not to produce mouse immunoglobulin (10% of SCID mice secrete some mouse immunoglobulin; no such mice were used in these experiments). Human immunoglobulin was quantitated by a 'sandwich' enzyme-linked immunosorbent assay (ELISA) using goat anti-human immunoglobulin (Cappel) as the capture reagent, pooled human serum of known immunoglobulin concentration as the standard, and peroxidase-conjugated goat anti-human immunoglobulin as the detection reagent. Microplates (Dynatech) pre-coated with the capture reagent were incubated for 3 h at room temperature with dilutions of SCID serum or the standard, extensively washed, and the detection reagent added. After peroxidase-catalysed colour development, the absorbance at 490 nm was quantitated on a Biotek microplate reader. Preliminary experiments showed that this assay did not detect mouse immunoglobulin in C.B-17 serum diluted 1:20, the starting dilution in the standard ELISA protocol. Sera from PBL-reconstituted SCID mice containing high levels of human immunoglobulin did not contain mouse immunoglobulin above the 1–5 µg ml⁻¹ levels found in conventional SCID mice.

for murine antigen-presenting cells cannot be entirely excluded. Further evidence of functional reconstitution was obtained from culturing the spleen or peritoneal cells of PBL-reconstituted SCID mice with tetanus toxoid, phytohaemagglutinin, or pokeweed mitogen. These antigens/mitogens stimulated increased thymidine incorporation in cells from most, but not all, recipients tested (data not shown), whereas non-reconstituted SCID mice failed to respond to these stimuli.

These experiments show that some functional human cells survived in SCID recipients. The number of surviving human cells and the representation of lymphocyte subsets was assayed by flow cytometry. Two groups of SCID recipients were analysed, one of which received 50×10^6 PBL from EBV-seropositive donors (Fig. 3a); the second received 10×10^6 PBL from the same donors (Fig. 3b). Two weeks after transfer in the first group and three weeks in the second group, human lymphoid cells accounted for 80–92% of the cells recovered from SCID spleens, and the absolute number of human cells per spleen in both groups ranged from 30×10^6 to 120×10^6 . This recovery of human cells in the spleen indicates both a migration from the peritoneal cavity to the spleen and expansion of cell number. We also detected human lymphocytes in the lymph nodes and peripheral blood of SCID mice, and the proportions of T and B cells were similar to those found in the spleen. We found few human cells in the peritoneal cavity until four weeks after transfer despite initial intraperitoneal injection. In the first group of SCID recipients receiving high numbers of PBL, there was an increased proportion of B cells (compared with fresh PBL) in all lymphoid organs at two weeks after transfer and this relative increase in B cells continued. The proportion of T cells

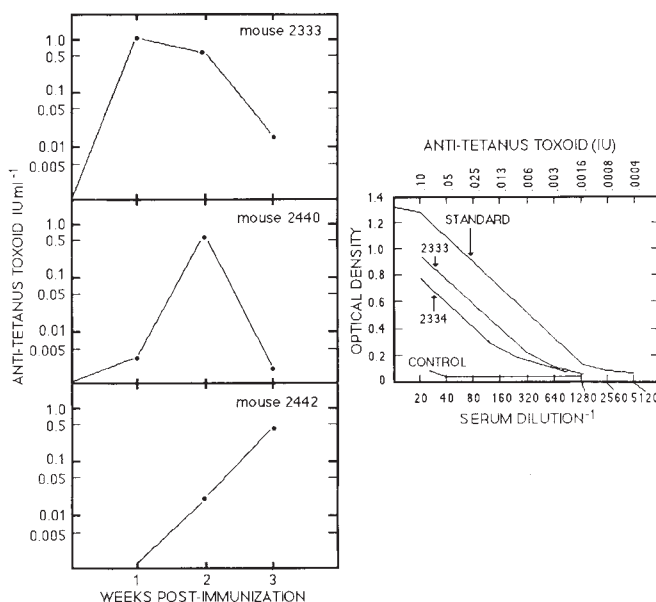


Fig. 2 Tetanus toxoid-specific antibody levels in representative SCID mice immunized with tetanus toxoid one (mice 2440 and 2442) or two (mice 2333 and 2334) weeks following reconstitution with 50×10^6 EBV+ PBL. The selected data represent the three patterns of response seen in 10 of 12 immunized animals.

Methods. PBL-reconstituted SCID mice were immunized i.p. with 100 µg trinitrophenyl(TNP)-conjugated tetanus toxoid in phosphate-buffered saline. Serum was collected before immunization and at weekly intervals thereafter. Antibodies to both TNP and tetanus toxoid were quantitated by ELISA but only data for tetanus toxoid-specific antibodies are reported here. TNP-specific antibody was detected following immunization, but the lack of appropriate control antibodies made quantitation difficult. The tetanus toxoid-specific ELISA was a direct binding assay in which tetanus toxoid (Wyeth Labs) was bound to microplates, and the binding of SCID serum to the plates compared with hyper-immune anti-tetanus globulin (Hypertet, Cutter) standardized to contain 250 IU ml⁻¹. After extensive washing, the bound control and standard antibody was detected with peroxidase-conjugated goat anti-human immunoglobulin as described in Fig. 1. This anti-human immunoglobulin did not cross-react with mouse immunoglobulin and was unable to detect tetanus toxoid-specific mouse antibodies elicited by secondary immunization of BALB/c mice. The right-hand panel illustrates the sensitivity of this ELISA and demonstrates that serum of SCID mice two weeks after immunization gave titration curves parallel to that of the standard. The detection limit of the assay is about 0.003 IU ml⁻¹, so all data reported are well above this limit with the exception of the one- and three-week points on mouse 2440. The two PBL-reconstituted SCID mice immunized at 20 weeks after transfer responded by producing 0.20 and 0.12 IU of antibody, respectively. For comparison, normal volunteers immunized with tetanus toxoid are reported to generate 8–16 IU of antibody using a similar ELISA⁸.

among surviving human cells decreased over time, and CD4⁺ cells decreased more than CD8⁺ T cells. By four weeks after transfer, small numbers of CD3⁺, CD4⁺, CD8⁺ (double negative) T cells appeared, and these then increased in many recipients. We also detected small numbers of CD5⁺ B cells in these recipients at four and eight weeks after transfer. We conclude that normal lymphocytes survive, expand and recirculate in SCID recipients, but that the relative proportion of subsets is altered and that normally minor subpopulations of double negative T cells and CD5⁺ B cells emerge. Whether this latter observation is related to early stages of lymphomagenesis is unclear⁹.

In contrast, a second group of SCID recipients of 10×10^6 PBL had a slightly slower expansion of human cell number, but a more normal lymphocyte subset profile (Fig. 3b). B lymphocytes were found in normal or slightly elevated proportions,

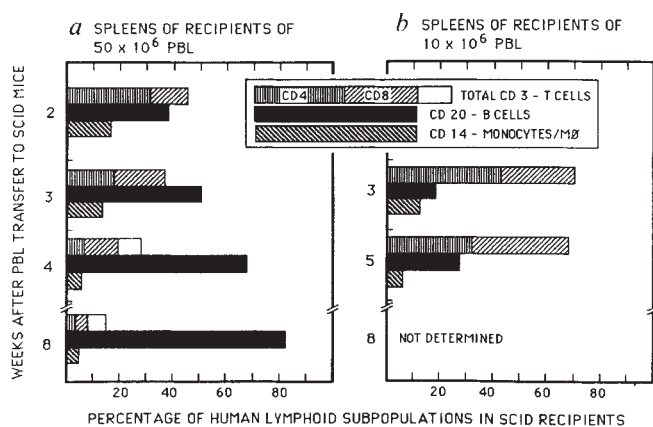


Fig. 3 Analysis by flow cytometry of human lymphoid populations recovered from the spleens of PBL-reconstituted SCID mice. *a*, Results for recipients of high PBL numbers. *b*, Results for recipients of low PBL numbers.

Methods. Spleens, peripheral lymph nodes, peritoneal cells and peripheral blood cells were collected from SCID recipients and single-cell suspensions prepared. The cells were stained for 30 min with fluorescein-conjugated monoclonal antibodies specific for the following markers: CD20 (B1, Coulter), CD3 (Leu 4, Becton-Dickinson), CD4 (Leu 3a, B-D), CD8 (Leu 2a, B-D), CD14 (MAC-3, B-D), murine immunoglobulin (Southern Biotech Assoc.) and murine Thy-1 (B-D). A biotinylated anti-CD5 antibody (B-D) and phycoerythrin-conjugated streptavidin (Biomed) were used to detect CD20, CD5⁺ cells. Staining was analysed on a Coulter EPICS 753 flow cytometer. The number of human cells staining with CD3, CD20 and CD14 antibodies accounted for 80–92% of the total analysed cells, so relatively few mouse cells were present in these cell suspensions and the normalization factor to standardize the data at 100% human cells was small. The absolute number of human cells recovered from SCID recipients varied with time and was dependent upon the initial cell inoculum. Recipients of 50×10^6 PBL showed early increases in T- and B-cell number, followed by major increases in B-cell number and a slow decrease in T-cell recovery. Recipients of 10×10^6 PBL showed slower increases in absolute cell recovery for both T and B cells, with $20\text{--}40 \times 10^6$ T cells and $8\text{--}20 \times 10^6$ B cells recovered in the spleen at five weeks post-reconstitution. No mouse T or B cells were detected except in one older long-term recipient that seemed to have a mouse T-cell lymphoma. Analysis of lymph node, peripheral blood and peritoneal cells gave similar results to those presented here. The normal distribution of lymphocytes and monocytes in PBL is similar to the distribution shown by recipients of 10×10^6 PBL three weeks after reconstitution.

CD3⁺ T cells were the most prevalent cell type found, and both CD4⁺ and CD8⁺ cells were present in substantial numbers. The ratio of CD4⁺:CD8⁺ cells initially was normal, but was reversed with time. The proportion of CD14⁺ (MAC-3⁺) cells diminished with time in these recipients. Although the data shown are for human cells recovered from the spleen, similar proportions of lymphocyte subsets were found in the peripheral blood, lymph nodes, and peritoneal cavity at five weeks post-reconstitution. The major differences between the recipients of high numbers of PBL and those of low numbers thus seems to reside in the extent of B-cell expansion and the absolute number of T cells surviving long term, with low dose recipients having fewer B cells and more T cells. These differences may be important for determining long-term survival versus lymphoma development.

These results are an important first step towards creating a better model system in which to test human immune responses. Nevertheless our observations in these mice pose important questions. We presume that functional human T and B lymphocytes in SCID mice are derived from long-lived recirculating mature cells, but we cannot exclude reconstitution by rare recirculating stem cells or progenitors. The normal regulatory interactions that lead to homeostasis in the intact immune system appear to be altered in PBL-reconstituted SCID mice, resulting

in spontaneous B-cell activation in all recipients and tumour formation in recipients of high numbers of EBV⁺ PBL. The role of human T cells responding to murine xenoantigens in these processes is undefined for the moment. The development of human B-cell lymphomas in SCID recipients of high, but not low, numbers of PBL is a provocative result, and is reminiscent of the development of EBV-positive lymphomas in human SCID patients receiving bone marrow transplants¹⁰. This outcome may reflect a low frequency of latent EBV infection in B cells¹¹, a rare transforming event, a B-cell expansion that is proportional to the strength of the xenogeneic GVH reaction by T cells, or a combination of all these factors. Nonetheless, this observation of rapid development of B-cell neoplasms provides an important new model for the study of human lymphomagenesis.

These observations raise many unresolved problems, but what seems to us most remarkable is that these xenografts were successful in the first place. With further manipulations, we believe that SCID mice reconstituted with human lymphoid cells can be made to mimic the intact human immune system even more closely. This would allow prediction of human immune responses to vaccines, for testing immunomodulators and for exploring pathogenic mechanisms in HIV infection and human neoplasia.

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Successful vaccination with a polyvalent live vector despite existing immunity to an expressed antigen

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A global vaccination strategy must take into account production and delivery costs as well as efficacy and safety. A heat-stable, polyvalent vaccine that requires only one inoculation and induces a high level of humoral and cellular immunity against several diseases is therefore desirable. A new approach is to use live microorganisms such as mycobacteria¹, enteric bacteria^{2,3}, adenoviruses⁴, herpesviruses^{5,6} and poxviruses⁷ as vaccine vectors. A potential limitation of live polyvalent vaccines, however, is existing immunity within the target population not only to the vector, but to any of the expressed antigens. This could restrict replication of the vector, curtail expression of antigens, and reduce

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the total immune response to the vaccine. Recently acquired immunity to vaccinia virus can severely limit the efficacy of a live recombinant vaccinia-based vaccine⁵, so a strategy involving closely spaced inoculations with the same vector expressing different antigens may present difficulties. We have constructed a recombinant vaccinia virus that expresses surface proteins from two diverse pathogens, influenza A virus haemagglutinin and herpes simplex virus type 1 (HSV-1) glycoprotein D. Mice that had recently recovered from infection with either HSV-1 or influenza A virus could still be effectively immunized with the double recombinant.

Influenza A virus and herpes simplex virus type 1 (HSV-1) are important human pathogens that have been adapted to lethally infect mice. Vaccination with recombinant vaccinia viruses expressing the influenza haemagglutinin (HA) prevents upper and lower respiratory tract infection with influenza A virus⁹⁻¹¹, and vaccination with recombinants expressing HSV-1 glycoprotein D (HSVgD) prevents both lethal and latent HSV infection in mice^{12,13}. It is possible to construct an infectious vaccinia recombinant which expresses multiple foreign antigens¹⁴. We have made a double recombinant vaccinia virus, vHA-gD, that expresses the HA of influenza A and the gD of HSV-1 on the surface of infected cells. This double recombinant virus protected mice against influenza and HSV infection as well as corresponding recombinants vHA and vgD, which express only a single gene, at all but the lowest vaccine dose tested (Fig. 1a and b). In general, the degree of protection correlated with antibody titres to influenza, as measured by haemagglutination inhibition (HI) assay, and to HSV, as measured by microneutralization assay¹³ (data not shown).

Because foreign antigens such as influenza HA and HSVgD are expressed on the surface of recombinant vaccinia virus-infected cells, existing immunity to one of these antigens might trigger killing of infected cells by antibody-dependent cytotoxic cells or antibody-dependent complement-mediated lysis. In addition, it is known that cells infected with recombinant vaccinia viruses are targets for cytotoxic T lymphocytes⁷, and accelerated cellular immunity could hinder replication of a recombinant virus. Association of influenza or HSV antigens with recombinant virus particles might also lead to direct neutralization of infectivity. In all cases, the immune response to both the vector and to novel antigens in a polyvalent vaccine would be greatly reduced.

To determine whether existing immunity to HSV would limit the efficacy of vHA-gD, six-week-old female BALB/c mice were given a sub-lethal dose of the mouse-adapted HSV-1 MacIntyre strain on day 0. On day 28, high serum neutralizing antibody titres to HSV (geometric mean titre > 1:300) were detected and mice were vaccinated with 10^8 , 10^6 or 10^4 plaque-forming units (PFU) of double recombinant vHA-gD or single recombinant vHA (ref. 15). On day 56, mice were challenged with 100 LD₅₀ (50% lethal dose) of influenza A/PR/8/34 virus. We found that 10^8 PFU of either vHA-gD or vHA produced almost total protection against a lethal challenge of influenza virus, 10^6 PFU protected most of the animals, and 10^4 PFU produced minimal protection (Fig. 1c). Thus, despite immunity to HSV-1, expression of HSVgD by the double recombinant did not impair vaccination against influenza. As expected, vaccination with a control vaccinia virus recombinant expressing the *Escherichia coli* β -galactosidase (v β gal)¹⁶ produced no protection against influenza (Fig. 1c).

To determine whether existing immunity to influenza would limit the efficacy of vHA-gD, mice were inoculated with a sub-lethal dose of mouse-adapted influenza A virus strain PR/8/34 on day 0. On day 28, high serum antibody titres to HA (mean HI titre > 1:128) were present and mice were vaccinated with 10^8 , 10^6 or 10^4 PFU of double recombinant vHA-gD or single recombinant vgD (ref. 13). On day 56, mice were challenged with 100 LD₅₀ of HSV-1 MacIntyre strain. 10^8 PFU of either vHA-gD or vgD produced complete protection against a lethal challenge of HSV-1, 10^6 PFU protected the majority of

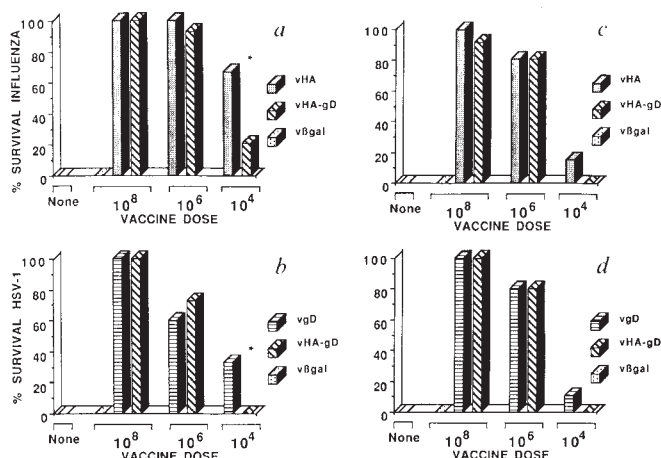


Fig. 1 Disease protection with a live polyvalent recombinant vaccine. (a, c) Mice that received placebo (a) or were infected (c) with a sublethal dose of HSV-1 (1×10^5 PFU, ~ 0.5 LD₅₀) on day 0 were immunized with the indicated vaccinia recombinant on day 28. On day 56, they were challenged with 100 LD₅₀ of influenza A/PR/8/34 mouse-adapted virus, and survival was recorded over a 21-day period. (b, d) Mice that received no virus (b) or were infected (d) with a sublethal dose of the influenza A/PR/8/34 virus ($10^{2.2}$ tissue culture infectious doses, ~ 0.03 LD₅₀) on day 0 were immunized with the indicated vaccinia recombinant on day 28. On day 56, they were challenged with 100 LD₅₀ of HSV-1 and survival recorded as above. Differences in protection obtained with single versus double recombinants were not statistically significant, except as indicated (* $P < 0.05$, Fisher's Exact test).

Methods. Double recombinant vHA-gD was constructed by first inserting the complete open reading frame for the influenza A/PR/8/34 haemagglutinin (provided by Peter Palese) into the *Sma*I site of pCF11, a plasmid vector that directs insertion of foreign genes plus the *E. coli* β -galactosidase into a non-attenuating deletion in the *Hind*III C region of the vaccinia genome²¹, yielding recombinant plasmid pYF1. CV-1 cells infected with recombinant vaccinia virus v52 (vgD) (ref. 13) were transfected with calcium phosphate-precipitated pYF1 plasmid DNA as described²², yielding double recombinant vYF1 (vHA-gD). Recombinant plaques were selected by the appearance of blue colour in the presence of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside $300 \mu\text{g ml}^{-1}$ (ref. 16). Correct expression of HA and gD in vHA-gD-infected cells was confirmed by immunoprecipitation and immunofluorescence (not shown). Purified vaccinia virus recombinants were diluted in phosphate buffered saline (PBS) and injected intraperitoneally. Groups of 13 to 20 six-week-old female BALB/c mice (NCI, Division of Cancer Treatment Animal Program, Frederick, Maryland) were used. Challenge with influenza A/PR/8/34 was by intranasal inoculation of lightly anaesthetized mice with virus delivered in a 0.1 ml volume of L15 (Liebowitz) Medium (Quality Biologicals). Mice were challenged with HSV-1 MacIntyre diluted in PBS and injected intraperitoneally. The LD₅₀ of influenza was determined by intranasal challenge (5 mice per log dilution of virus), and the LD₅₀ of HSV-1 was determined by intraperitoneal challenge (5 mice per log dilution of virus). Vaccinia recombinants²², mouse-adapted influenza A/PR/8/34 (ref. 23), and mouse-adapted HSV-1 MacIntyre²⁴ were purified and titred as described.

animals, and 10^4 PFU produced minimal protection (Fig. 1d). Vaccination with vaccinia control v β gal produced no protection against HSV-1 (Fig. 1d). Thus, despite previous immunity to influenza virus, expression of HA by the double recombinant did not impair vaccination against HSV-1.

Analysis of antibody titres in vaccinated animals was consistent with disease protection data and previous immunity to HSV-gD or influenza HA. Antibody titres to influenza HA were high with 10^8 PFU, intermediate with 10^6 PFU, and low with 10^4 PFU of vHA or vHA-gD (Fig. 2a). Single recombinant vHA produced a slightly higher anti-influenza virus antibody titre

than the double recombinant at a dose of 10^8 PFU, but anti-influenza titres were comparable at other doses. On day 56, high levels of anti-HSV-1 antibodies were detected by enzyme-linked immunosorbent assays (ELISA) in all animals infected at day 0 with HSV-1 (Fig. 2b), but those animals vaccinated with vHA-gD produced substantially higher anti-HSV antibodies than animals vaccinated with vHA, indicating a significant immunological boost. This boosting effect was confined to the higher doses (10^8 and 10^6 PFU) of the double recombinant and was not present when the vaccine dose was dropped to 10^4 PFU.

High levels of anti-influenza antibodies were detected by ELISA on day 56 in all animals infected at day 0 with influenza virus (Fig. 2c), but those animals vaccinated with vHA-gD produced substantially higher anti-influenza antibodies than animals vaccinated with vgD, once more indicating a significant immunological boost which was again confined to the higher doses (10^8 and 10^6 PFU) of the double recombinant. In animals previously infected with influenza A, antibody titres to HSV were high with 10^8 PFU, intermediate with 10^6 PFU, but were not significantly greater than control (v β gal-vaccinated mice) with 10^4 PFU of vgD or vHA-gD (Fig. 2d). Anti-HSV titres were equivalent at all doses of either vgD or vHA-gD tested.

The use of recombinant live virus vectors would raise problems not encountered with classical live or polyvalent killed vaccines. For example, we found that recently acquired immunity to a recombinant vaccinia virus expressing the hepatitis B surface antigen or influenza HA limited the subsequent immune response produced by vaccinating with a recombinant vaccinia virus expressing HSVgD (ref. 8). The failure to generate a neutralizing HSV antibody response or to protect against lethal and latent HSV infections is a direct consequence of the acquired immunity to vaccinia virus, which restricted replication of the vector. The most effective live vector vaccine strategy might therefore require simultaneous rather than successive immunizations against multiple antigens. This could be accomplished either with a cocktail of single recombinant vaccines or a polyvalent recombinant. The polyvalent vector would be more convenient to mass-produce, formulate and administer than a vaccine mixture. It is likely that some individuals could already be immune to one or more of the antigenic components of a polyvalent vaccine and so it seemed important to explore the possibility that existing immunity to these antigens might also limit replication of the recombinant vector and thereby compromise the effectiveness of the vaccine.

To mimic a natural situation, one group of mice was sublethally infected with HSV-1 and another with influenza virus; both groups were then vaccinated 28 days later with a double recombinant that expressed both antigens. If vaccination with the double recombinant were less effective than vaccination with the corresponding single recombinants, as judged by either protection or antibody production, this would indicate a serious flaw in polyvalent recombinant vaccine strategy. We found, however, that the existing immunity to HSV-1 or influenza did not adversely affect the vaccination with the double recombinant vector. Before vaccination, these animals were shown to have substantial immunity to the corresponding foreign antigens expressed by the double recombinant, as indicated by influenza HI and HSV neutralization titres. The influenza HI assay measures anti-HA antibody¹⁷; BALB/c mice infected with HSV-1 produce anti-gD antibodies¹⁸, and these antibodies result in virus neutralization^{13,19}. Furthermore, a significant increase in antibody production to the recognized antigen provided by the double recombinant proved that the animals were primed for an anamnestic response, which could only occur after previous immunity to HA or gD (Fig. 2b and c). Nonetheless, this boost was insufficient to limit replication of the vector. Similar levels of replication of double and single recombinants were confirmed by anti-vaccinia virus antibody titres, which varied with vaccine dose and were nearly identical with either vHA, vgD, or vHA-gD (Fig. 2 legend, data not shown).

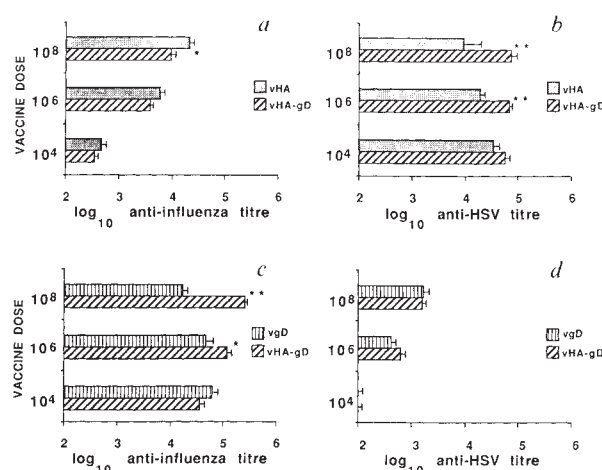


Fig. 2 Immunogenicity of double and single recombinants in mice previously infected with HSV-1 (a, b) or influenza virus (c, d). On day 0, female BALB/c mice were infected with HSV-1 (a, b) or influenza virus (c, d), and on day 28 were vaccinated with the indicated inoculum of recombinant vaccinia virus (see Fig. 1). Serum (day 56) ELISA antibody titres (reciprocal mean log₁₀ + one standard error) from groups of 16 to 20 mice are shown. Statistically significant differences between samples are indicated (Student's two-tailed *t*-test, applied to double versus single recombinants at the same dose only, **P* < 0.05, ***P* < 0.01). Pre-immune titres to vaccinia, influenza virus, and HSV were <1:100 in all cases (not shown). Post-infection antibody titres (log₁₀ reciprocal mean ELISA titres, day 28) were 2.70 ± 0.06 for HSV-1 (*n* = 23) and 4.36 ± 0.10 for influenza A virus (*n* = 19). Anti-influenza titres on day 56 from control mice infected with HSV-1 and vaccinated with 10^8 PFU of v β gal (not shown) were <1:100, and anti-HSV titres from these mice were equivalent to those of mice vaccinated with 10^8 PFU of vHA (b); anti-influenza titres on day 56 from control mice infected with influenza virus and vaccinated with 10^8 PFU of v β gal (not shown) were equivalent to those of mice vaccinated with 10^8 PFU of vgD (c), and anti-HSV titres from these mice were <1:100.

Methods. ELISAs for influenza A virus¹⁷, HSV-1²⁴, and vaccinia virus²⁵ were modified slightly from previously reported protocols. For vaccinia virus, microtitre plates were coated overnight at 4 °C with purified WR strain vaccinia virus, $\sim 5 \times 10^5$ PFU per well, diluted in carbonate-bicarbonate buffer (15 mM Na₂CO₃ + 35 mM NaHCO₃, pH 9.6); serial 4-fold dilutions of serum were incubated with a 1:2,000 dilution of rabbit anti-mouse immunoglobulin G antiserum (Cappel); after washing, plates were incubated with a 1:16,000 dilution of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Miles Laboratories). After incubation with alkaline phosphatase substrate for 1 h, optical density of wells at 405 nm was read on a Vmax Kinetic Microplate Reader (Molecular Device Corp., Palo Alto, California). ELISAs for influenza virus and HSV-1 were similar, but the amount of antigen used to coat wells was ~ 8 haemagglutination units per well of purified influenza A/PR/8/34, or $\sim 10^5$ PFU per well of purified HSV-1, MacIntyre strain.

Both of the antigens expressed by vHA-gD are important targets for humoral immunity in mice¹⁰⁻¹³, and HA has been shown to be a target for cytotoxic T cells as well¹⁵. Although cellular and humoral responses appear to be involved in host defense against poxviruses²⁰, immunity to foreign antigens expressed by a bivalent vector does not appear to readily suppress vector replication. These results should encourage further investigation and development of polyvalent recombinant vaccines.

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A naturally immunogenic virion-associated protein specific for HIV-2 and SIV

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The genomic organization of HIV-1 and the family of HIV-2 and SIV viruses¹⁻⁴ is similar. However, there is an open reading frame, *orf-x*, that is present in HIV-2 and SIV, but not in HIV-1^{3,4}. The extent of protein sequence conservation in *orf-x* between HIV-2_{ROD} and SIV_{MAC}⁴ suggests that this open reading frame encodes a gene that may be important for infectivity or replication. Here, we show that the *orf-x* products of SIV_{MAC} and HIV-2_{SBL-6669} are virion-associated and that the introduction of a premature stop codon into *orf-x*, did not abrogate virus infectivity and replication *in vitro*. Antibody reactivity to the *orf-x* product was detected in 35 of 42 HIV-2 positive serum samples and 11 of 52 SIV seropositive monkeys. No such antibodies were detected in HIV-1 positive donors, blood donors seronegative for both HIV-2 and HIV-1, or SIV seronegative monkeys. This suggests that *orf-x* is dispensable for *in vitro* replication of SIV_{MAC} and that the *orf-x* gene product of HIV-2 or its antibody can be used to distinguish HIV-2 from HIV-1 infection.

A plasmid, pX-2, was constructed to produce SIV_{MAC} *orf-x*-encoded peptide in *Escherichia coli* (Fig. 1a). This vector is modified from plasmids pUC-18 and pXVR⁵, and contains a 3.1 kb *StuI*-*PstI* fragment from an SIV infectious clone, pBK-28^{6,7}. DNA sequence analysis (Fig. 1b) showed the *orf-x* sequence in pX-2 was in the same reading frame as the preceding *v-ras*^H sequence. Upon induction by isopropyl-β-D-thiogalactopyranoside (IPTG), a *v-ras*^H/*orf-x* fusion protein with a rela-

tive molecular mass (M_r) of about 27,000 (27 K) was detected (Fig. 1c). This protein was recognized by goat antibodies to *v-ras*^H-encoded p21 (Fig. 2a), showing that the correct reading frame for the *orf-x* sequence was being used. The size of the recombinant *orf-x* protein was consistent with predictions, and it was therefore unlikely that read-through had occurred. Antibody reactivity to the recombinant *orf-x* protein was detected in 35 of the 42 HIV-2 seropositive West Africans tested. None of the 41 HIV-1 antibody-positive human sera from donors from the United States and West Africa nor any of the 31 United States and West African blood donor sera that were negative for antibodies to HIV-1 and HIV-2 reacted with this protein. Antibody binding reactivity of representative sera is shown in Fig. 2a. In contrast with the high antibody prevalence among people infected with HIV-2, only 11 of the 52 SIV-seropositive monkeys studied had detectable antibody reactivity to the recombinant *orf-x* protein. Figure 2b shows antibody binding profiles of representative monkey sera. All sera which reacted with the *v-ras*^H/*orf-x* fusion protein lacked antibody reactivity to *v-ras*^H p21 (Fig. 2c) expressed by pXVR, indicating that antibody reactivity was directed to the *orf-x* portion of the fusion protein.

A protein of M_r of about 12 K was identified as the native *orf-x* product in SIV_{MAC}-infected cells using radio-immunoprecipitation (Fig. 3a). This protein, called p12^x here, was detected in Hut-78 cells infected by cell-free virions. These were derived from Hut-78 cells transfected with plasmid pBK-28 (refs 6, 7). As shown in Fig. 3a, a post-immune goat serum directed to the recombinant *orf-x* protein reacted with p12^x; the pre-immune serum from the same goat did not. Cross-reactivity to SIV_{MAC} p12^x detected by a representative HIV-2-positive human serum is also shown in Fig. 3a. Human sera negative for HIV-1 and HIV-2 did not precipitate p12^x (data not shown).

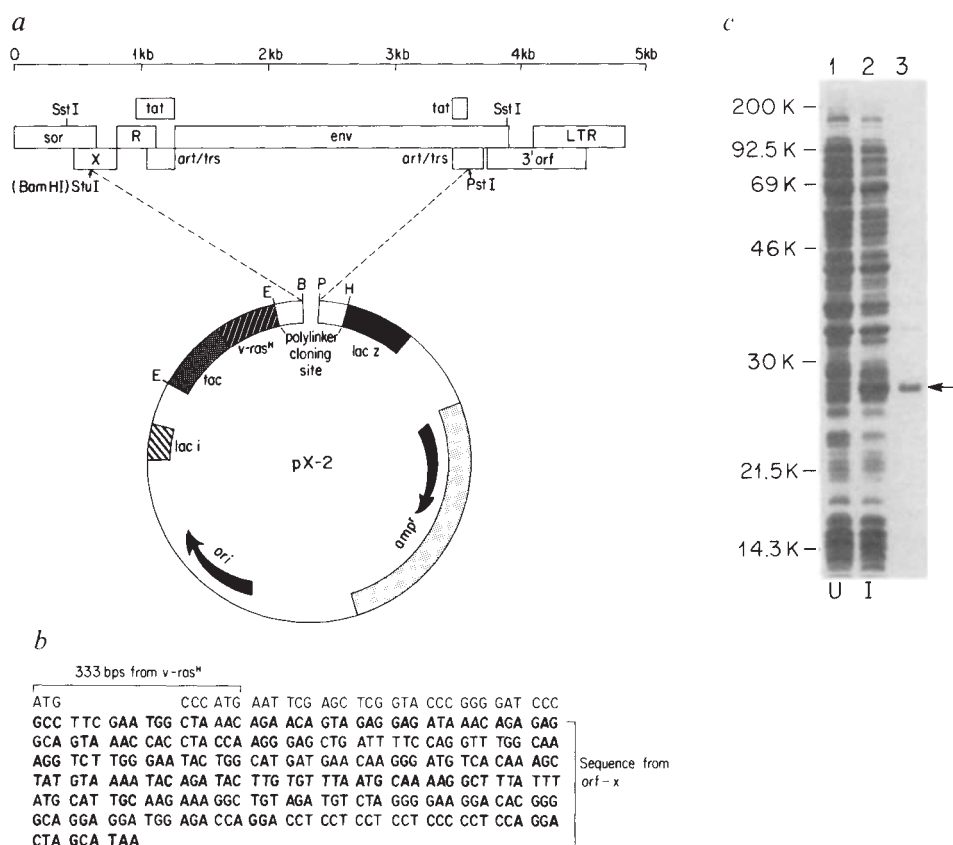
The viral specificity of p12^x was confirmed by its absence in Hut-78 cells infected by a mutant virus with a truncated *orf-x* (Fig. 3a). The absence of p12^x in the viral lysates prepared from cells infected with the mutant virus, provided further evidence of viral specificity (Fig. 3a). The *orf-x* mutant clone designated pBK-x, was constructed by inserting a *XbaI* linker d(TGCTCTAGAGCA) at the *StuI* site within the *x* coding sequence (Fig. 1). This linker contains the stop codon TAG which causes premature termination in *orf-x* and allows the synthesis of the first 20 amino acids only. The mutant pBK-x contains an additional *XbaI* site and lacks one *StuI* site compared with wild-type pBK-28. These differences were readily scored by Southern blot analysis (data not shown).

The *orf-x*-encoded protein is also virion-associated. This was first suggested by the detection of p12^x in the viral lysate by immunoblotting (Fig. 3a). Further analysis was performed with sucrose-gradient-banded SIV_{MAC} (Fig. 3b). Virions present in fractions 4 and 5 which contained peak reverse transcriptase activity were pooled and subjected to immunoblotting analysis. As shown in Fig. 3b, p12^x was specifically recognized by both the post-immune goat serum directed to the recombinant *orf-x* protein and an SIV-seropositive monkey serum, but not by the pre-immune goat serum, and an SIV-seronegative monkey serum. These results were consistent with our conclusion that p12^x is associated with cell-free virions.

Immunoblotting analysis was used to identify the protein encoded by *orf-x* of HIV-2 in viral lysates prepared from HIV-2_{SBL-6669} producer cells⁸⁻⁹. A protein with a relative molecular mass of about 16 K was specifically recognized by the post-immune goat serum directed to SIV_{MAC} *orf-x* recombinant protein, but not by the pre-immune goat serum (Fig. 3c). Similarly the *orf-x* product of HIV-2_{SBL-6669} was recognized by HIV-2 positive human sera, but not by HIV-1 positive human sera or human sera negative for both HIV-1 and HIV-2. Cross-reactivity to HIV-2_{SBL-6669} *orf-x* protein by SIV seropositive monkeys was also observed. Antibody binding profiles of representative sera are shown in Fig. 3c. Similar results were

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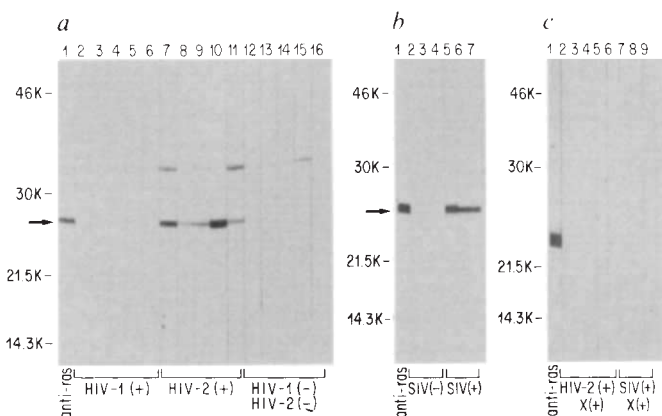
Fig. 1 *a*, Bacterial expression plasmid pX-2. This plasmid is modified from plasmid pUC-18 and contains part of plasmids pXVR⁵ and pBK-28^{6,7}. It contains an *Eco*RI (E) fragment which has a *tac* promoter linked to the first 333 nucleotides of *v-ras*^H. Downstream from the *v-ras*^H coding sequence is the pUC-18 polylinker cloning site. The 3.5 kb *Sst*I fragment of pBK-28 was excised and subcloned into pUC-18. This subcloned plasmid was digested with *Bam*HI and ligated with a *Bam*HI (B) linker, d(CGCGATCCG). Plasmid DNA with the *Bam*HI linker was digested with *Bam*HI and *Pst*I (P). The 3.1-kb *Bam*HI-*Pst*I fragment which includes most of the *orf-x* coding sequence was inserted into the polylinker cloning site. *b*, DNA sequence analysis of pX-2. The nucleotide sequence of a 0.6-kb *Hind*III DNA fragment starting in the *v-ras*^H gene and ending in the R region of pBK-28 was determined by the dideoxy nucleotide sequencing method (USB, Cleveland, Ohio). *c*, Polyacrylamide gel electrophoresis of recombinant *orf-x* protein. Lane 1, uninduced whole bacterial lysates (U); lane 2, isopropyl- β -D-thiogalactopyranoside (IPTG)-induced bacterial lysates (I); lane 3, partially purified *orf-x* recombinant protein as indicated by the arrow.



Methods. Samples of an overnight culture of X-90 (200 μ l)¹¹ bearing pX-2 was inoculated into 10-ml Luria-Bertani media containing 50 μ g ml⁻¹ ampicillin, and the cells were grown to an A_{550} of 0.3. Cultures (5 ml) were induced with 5 mM IPTG and the remainder was used as an uninduced control. After 3 h at 37 °C, the cells were collected by centrifugation at 3,000 r.p.m. (Beckman AccuSpin FR) for 10 min and washed once with 50 mM NaCl, 10 mM Tris-Cl pH 7.5. Cells were lysed in 500 μ l of Laemmli sample buffer¹² and boiled for 2 min before loading on the gel. Partial purification procedure for *orf-x* recombinant protein was modified from that of Pallas *et al.*¹¹ All the steps were the same except that the final wash was carried out in 8 M urea, with 6 ml used for each 250 ml bacterial culture. Samples were analysed on 15% polyacrylamide gels using the Laemmli buffer system¹² and stained with Coomassie blue.

Fig. 2 Immunoblotting of partially purified *orf-x* recombinant protein (*a* and *b*). *a*, Lane 1, goat anti-*ras* serum; lanes 2-6, sera from HIV-1 positive donors; lanes 7-11, sera from HIV-2 positive West Africans; lanes 12-16, sera from blood donors seronegative for both HIV-1 and HIV-2. *b*, Lane 1, goat anti-*ras* serum; lanes 2-4 SIV seronegative monkey sera; lanes 5-7, SIV seropositive monkey sera. The arrow indicates the 27 K *orf-x* recombinant protein. *c*, Immunoblotting of partially purified *ras* protein. Lane 1, goat anti-*ras* serum; lanes 2-6, the HIV-2 positive human sera in lanes 7-11 of *a*; lanes 7-9, the SIV positive sera in lanes 5-7 of panel *b*.

Methods. Procedures for immunoblotting were as described previously¹³. The goat anti-*ras* serum used in lane 1 was raised against a recombinant protein containing the first 111 amino acids of *v-ras*^H. Approximately 0.8 mg of polyacrylamide-gel-purified protein was used for initial immunization. Booster shots were given on days 14 and 21 with approximately 0.4 mg of the same material. A serum collected 28 days after immunization was used in this study. Pre-immune serum had no reactivity to the *orf-x* recombinant protein (data not shown). Procedures for partial purification of *orf-x* recombinant protein were followed for the purification of *ras* protein expressed by plasmid pXVR. All HIV-2 positive sera were found to have more binding reactivity to the protein gp120 encoded by the *env* gene of SIV_{MAC} than that of HIV-1_{IIIB} (M. J. Chou, unpublished data).



obtained with another HIV-2 strain, HIV-2_{NIH} (ref. 9 and data not shown). The apparent relative molecular masses of the *orf-x* gene products of SIV_{MAC} and HIV-2 viruses is similar to that of SIV_{Mac}¹⁰ and in agreement with the coding potential of the *orf-x*, suggesting that the *orf-x* product does not undergo extensive post-translational modifications.

In vitro, the *orf-x* gene appears to be dispensable for SIV_{MAC} replication and infectivity as cell-free virions and SIV proteins other than p12^x were readily detected in Hut-78 cells infected by the *orf-x* mutant virus (Fig. 3a). We did not find a significant difference in the amount of virion proteins produced by cells infected with the wild-type virus and the *orf-x* mutant virus nine

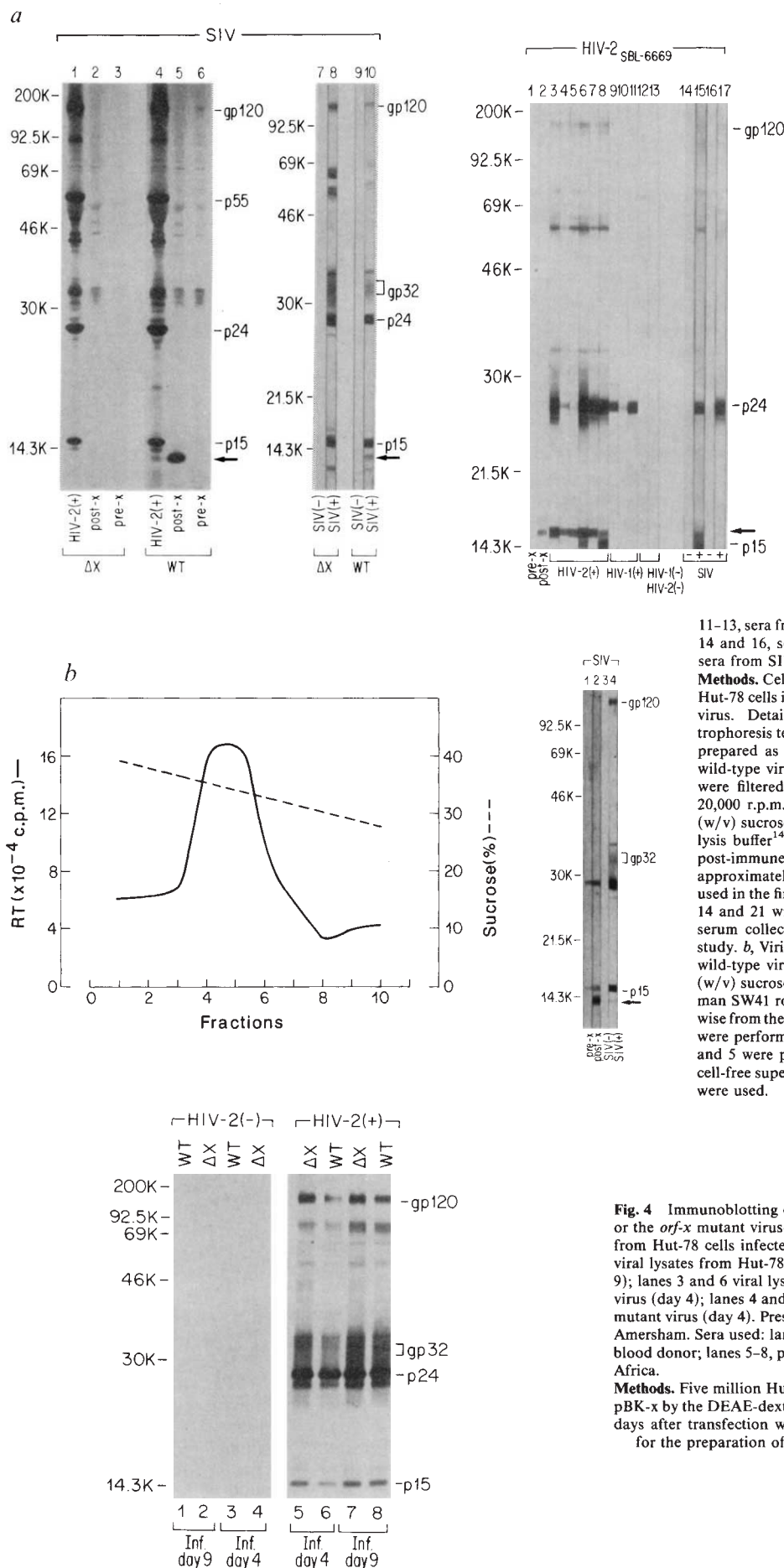


Fig. 3 *a*, Detection of p12* in SIV_{MAC}-infected Hut-78 cells by radio-immunoprecipitation (RIP) and immunoblotting. RIP analysis: lanes 1-3, cell lysates from cultures infected by the mutant virus (Δx); lanes 4-6, cell lysates from cultures infected by the wild-type virus (WT); lanes 1 and 4, serum from an HIV-2 positive donor; lanes 2 and 5, post-immune goat serum to the recombinant *orf-x* protein; lanes 3 and 6, pre-immune serum from the same goat. The arrow indicates p12*. Immunoblotting: lanes 7-8, viral lysates from cultures infected by the mutant virus; lanes 9-10, viral lysates from cultures infected by the wild-type virus; lanes 7 and 9, SIV negative monkey sera; lanes 8 and 10, SIV positive monkey sera. *b*, Detection of p12* in sucrose-gradient-banded SIV_{MAC}. Left: reverse transcriptase (RT) activity of sucrose-gradient-banded SIV_{MAC}. Right: immunoblotting of SIV_{MAC} pooled from fractions 4 and 5. Sera tested were: lane 1, pre-immune goat serum; lane 2, post-immune goat serum to the recombinant *orf-x* protein; lane 3, SIV seronegative monkey serum; lane 4, SIV seropositive monkey serum. The arrow indicates p12*. Background binding to p24 and p15 by the pre-immune goat serum was observed. *c*, Immunoblotting of HIV-2_{SBL-6669} *orf-x* protein in viral lysates. Lane 1, pre-immune goat serum; lane 2 post-immune goat serum to the recombinant *orf-x* protein; lanes 3-8, sera from HIV-2 positive donors; lanes 9-11, sera from HIV-1 positive blood donors; lanes 11-13, sera from HIV-2/HIV-1 seronegative blood donors; lanes 14 and 16, sera from SIV negative monkeys; lanes 15 and 17, sera from SIV positive monkeys.

Methods. Cell lysates were prepared from [³⁵S]cysteine-labelled Hut-78 cells infected with the wild-type virus or the *orf-x* mutant virus. Details of the RIP/SDS polyacrylamide gel electrophoresis techniques have been described¹⁴. Viral lysates were prepared as follows: 60 ml of cell-free supernatants from the wild-type virus or the *orf-x* mutant virus-infected Hut-78 cells were filtered through 0.45 μ m filter units and centrifuged at 20,000 r.p.m. (Beckman SW 28 rotor) for 2 h through a 20% (w/v) sucrose cushion. Virus pellets were lysed in 200 μ l RIPA lysis buffer¹⁴ and subjected to immunoblotting¹³. To generate post-immune goat serum to the *orf-x* recombinant protein, approximately 0.8 mg of polyacrylamide gel purified protein was used in the first immunization. Booster shots were given on days 14 and 21 with approximately 0.4 mg of the same material. A serum collected 28 days after immunization was used in this study. *b*, Virions prepared from cell-free culture supernatant of wild-type virus-infected Hut-78 cells were layered on 20-50% (w/v) sucrose gradients and centrifuged at 35,000 r.p.m. (Beckman SW41 rotor) for 6 h. Fractions (1 ml) were obtained dropwise from the bottoms of the tubes. Reverse transcriptase assays¹⁵ were performed on 72 μ l portions of each fraction. Fractions 4 and 5 were pooled and centrifuged at 35,000 r.p.m. for 1 h. *c*, cell-free supernatants (30 ml) from HIV-2_{SBL-6669} producer cells were used.

Fig. 4 Immunoblotting of virion proteins produced by the wild-type virus or the *orf-x* mutant virus-infected Hut-78 cells. Lanes 1 and 8, viral lysates from Hut-78 cells infected with the wild-type virus (day 9); lanes 2 and 7 viral lysates from Hut-78 cells infected with the virus mutant in *orf-x* (day 9); lanes 3 and 6 viral lysates from Hut-78 cells infected with the wild-type virus (day 4); lanes 4 and 5 viral lysates from Hut-78 cells infected with the mutant virus (day 4). Prestained relative molecular mass markers were from Amersham. Sera used: lanes 1-4, serum from an HIV-1 and HIV-2 negative blood donor; lanes 5-8, pooled sera of six HIV-2 positive donors from West Africa.

Methods. Five million Hut-78 cells were transfected with 5 μ g of pBK-28 or pBK-x by the DEAE-dextran method⁶. Cell-free supernatants collected nine days after transfection were used to infect fresh Hut-78 cells. Procedures for the preparation of viral lysates and immunoblotting as in Fig. 3.

days after infection using immunoblots. However, at four days after infection the cells infected with the wild-type virus appeared to produce fewer virions (Fig. 4). The observations that wild-type virus apparently replicated slightly slower than the *orf-x* mutant virus are compatible with the hypothesis that the *orf-x* gene has a negative, and therefore dispensable, role in the replication cycle of SIV_{MAC} .

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Note added in proof: The proposed nomenclature for the *x* gene is *vpx* (ref. 16).

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MHC polymorphism pre-dating speciation

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Two features distinguish the polymorphism of the major histocompatibility complex (MHC) loci from that of other loci: its high diversity and the large genetic distance between MHC alleles^{1,2}. More than 100 alleles exist in natural populations in the mouse at each of the functional class I and class II alleles, all alleles occurring at frequencies that cannot be explained by recurrent mutations^{2,3}. Some of the alleles differ by approximately 70 nucleotides in the coding region alone and some of the products of the allelic genes differ by more than 50 amino acids⁴. It has generally been assumed that these differences accumulated after species inception. Here, we present evidence for an alternative explanation of the origin of MHC polymorphism: a large part of the MHC polymorphism pre-dates speciation and is passed on from species to species^{5,6}. We describe allelic differences that must have arisen before the separation of mice and rats from a common ancestor more than 10 million years ago.

The major histocompatibility complex consists of two classes of loci, class I and class II, whose function is to provide self-context for the recognition of foreign antigens by T lymphocytes⁷. There are four functional class II MHC loci in the mouse: A_α , A_β , E_α and E_β . The proteins encoded in the alleles of the A_β locus fall into two groups defined by reactions with monoclonal antibodies⁸. One group reacts with antibodies specific for the antigenic determinant H-2A.m25; the other with antibodies specific for the determinant H-2A.m27. No protein has been found that reacts with both antibodies and only one protein reacts with neither of the two antibodies. Individuals can, however, express both determinants if they are heterozygous for the two corresponding alleles.

This serological reactivity pattern correlates with a specific structural feature of the proteins in the two groups. The H-2A.m27-positive proteins have two amino-acid residues deleted, at positions 65 and 67 in the first extracellular domain (β 1,

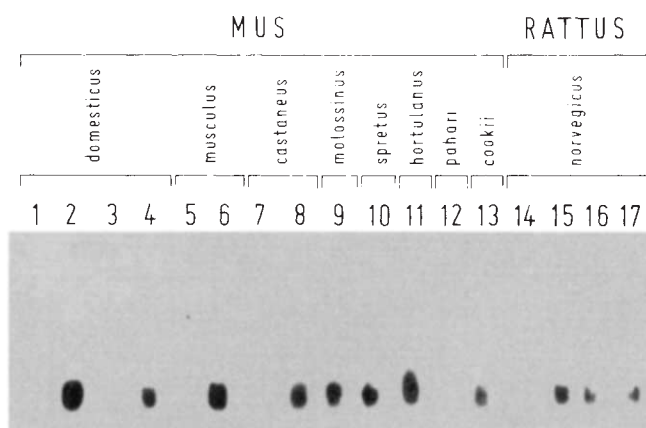


Fig. 1 An example of Northern blot hybridization between the A_β^A -specific oligonucleotide probe and RNA isolated from A_β^A and A_β^B mouse inbred strains and from other H-2A.m27 individuals belonging to several other species of the genus *Mus* and from rat inbred strains. The lanes correspond to the following strains: 1, C57BL/10; 2, B10.BR; 3, B10.D2; 4, B10.S; 5, PLD826; 6, MSW251; 7, B10.CAS2; 8, B10.CAS1; 9, B10.MOL1; 10-13, wild individuals; 14, LEW.BN; 15, BUF; 16, LEW.DA; 17, LEW.

Methods. The oligonucleotide was synthesized as described¹⁹. The RNA was prepared and Northern blots were obtained as previously described²⁰.

encoded in the second exon). In most of the H-2A.m25-positive proteins, these positions are occupied by Pro and Ile, respectively. The codon at position 66 is TAC (coding for Tyr) in the group with the deletions (the A_β^A group) and GAG (coding for Glu) in the group without the deletion (the A_β^B group). These nine nucleotides in the A_β gene therefore seem to code for the determinants H-2A.m25 and H-2A.m27. This supposition is supported by analysis of the $A_\beta^{\text{bm}12}$ mutant. The $A_\beta^{\text{bm}12}$ allele differs from the standard A_β^B allele in three nucleotides, one of which occurs in codon 67 (ATC to TTC; see ref. 9). The A_β^B protein reacts with the H-2A.m25-specific antibody, unlike the $A_\beta^{\text{bm}12}$ protein, which is the only H-2A.m25-negative, H-2A.m27-negative mouse A_β protein detected.

The two serological groups of the A_β protein represent true polymorphism within the house mouse species. Serological typing of wild mice reveals the presence of both H-2A.m25-positive and H-2A.m27-positive proteins in natural populations, at frequencies of 63.5% and 36.5%, respectively⁸. The correlation between the serological pattern and the presence or absence of

Table 1 Correlation between serological reactivity and the hybridization with the deletion-specific oligonucleotide

A_β allele	Strains	Reactivity of Mab specific for		Hybridization
		H-2A.m25	H-2A.m27	
	<i>M. domesticus</i>			
<i>b</i>	C57BL/10Sn, B10.STA62	+	-	-
<i>d</i>	B10.D2, B10.KEA2	+	-	-
<i>f</i>	B10.M	-	+	+
<i>j</i>	B10.WB	-	+	+
<i>k</i>	B10.BR, B10.STC90*, B10.SNA70	-	+	+
<i>p</i>	B10.P, B10.KEA5, B10.CAA2, B10.STC77, B10.CHR51	+	-	-
<i>q</i>	B10.Q	+	-	-
<i>r</i>	B10.RIII	-	+	+
<i>s</i>	B10.S, T7WF, B10.KPB128, B10.GAA37	-	+	+
<i>u</i>	B10.PL, B10.NZW	-	+	+
<i>v</i>	B10.SM, B10.KPA42, B10.KPA132, B10.SNA57, B10.DRB62, B10.WOA105	+	-	-
<i>w3</i>	B10.SAA48	+	-	-
<i>w4</i>	B10.GAA20	-	+	+
<i>w7</i>	B10.WOA1, B10.WR7	-	+	+
<i>w13</i>	B10.STA10, B10.STA12, B10.LIB55, B10.STA39	+	-	-
<i>w16</i>	B10.BUA1, B10.LIB18, B10.BUA16, B10.BUA19, B10.KPA44	-	+	+
<i>w2</i>	EDY589	-	+	+
<i>w30</i>	GPC882	-	+	+
<i>w31</i>	GPC183, CRO437*	-	+	+
<i>w36</i>	LGN925, BNK761, ERP1465	+	-	-
<i>w37</i>	CRO435	-	+	+
	<i>M. musculus</i>			
<i>w36</i>	PLD826	+	-	-
<i>w38</i>	MSW51	-	+	+
<i>w31</i>	BRU382	-	+	+
<i>w61</i>	BRW942	-	+	+
<i>w17</i>	B10.CAS2 (<i>M. castaneus</i>)	+	-	-
<i>w23</i>	B10.CAS1 (<i>M. castaneus</i>)	-	+	+
<i>k</i>	B10.MOL1 (<i>M. molossinus</i>)	-	+	+
†	Wild (<i>M. molossinus</i>)	+	-	ND
†	Wild (<i>M. spretus</i>)	-	+	+
†	Wild (<i>M. spretus</i>)	+	+	+
†	Wild (<i>M. hortulanus</i>)	-	+	+
†	Wild (<i>M. hortulanus</i>)	+	-	ND
†	Wild (<i>M. platythrix</i>)	-	+	ND
†	Wild (<i>M. cooki</i>)	-	+	+
†	Wild (<i>M. pahari</i>)	-	+	-
†	Wild (<i>M. caroli</i>)	-	+	ND

Mab, monoclonal antibody; ND, not done. * Variant of the defined allele. † Unknown allele.

the two deletions had, however, been demonstrated only on a small sample of alleles that have been isolated from inbred strains and sequenced. To determine whether this correlation will also hold for a large sample of alleles extracted from wild mice, we used an oligonucleotide probe to test for the presence of the two deletions. The probe corresponds to the noncoding DNA strand of the A_β^A allelic group (codons 64, 66, 68, 69, 70, 71; sequence GTCATGGACCTCGCTT). The probe was then used to distinguish the A_β^A from the A_β^V alleles by hybridization to Northern filters. Typing of inbred strains carrying sequenced A_β alleles confirmed that the probe was specific for A_β^A alleles: the probe hybridized with all alleles that had the two deletions but with none of the alleles that lacked the deletions (Fig. 1 and Table 1). Typing of an additional 45 A_β alleles, most of them extracted from wild mice, revealed a perfect correlation between the ability to hybridize with the probe (presence of deletions) and being H-2A m27-positive. In the entire sample of 53 A_β alleles tested, all strains expressing the H-2A.m27 determinant hybridized with the probe; all those expressing the H-2A.m25 determinant did not (Table 1). We conclude, therefore, that the oligonucleotide probe is specific for the two deletions and also for the H-2A.m27 determinant.

Earlier serological typing of mouse species other than *Mus domesticus* and *M. musculus* revealed the presence of the H-2A.m25/H-2Am27 polymorphism in *M. castaneus*, *M.*

molossinus, *M. spretus* and *M. hortulanus*. In each of these species, some individuals type H-2A.m25-positive and others H-2A.m27-positive. Single individuals tested from *M. pahari*, *M. caroli*, *M. platythrix* and *M. cooki*⁸ typed as H-2A.m27-positive. When tested with the oligonucleotide probe, RNAs from all but one of the H-2A.m27-positive individuals hybridized with it (Fig. 1; Table 1; the anomalous result obtained with *M. pahari* remains to be explained). These results suggest that the same deletion polymorphism found in *M. domesticus*/*M. musculus* exists also in other species of the genus *Mus*, some of which are believed to have been separated from each other for 2-5 million years¹⁰.

We examined several inbred strains of the rat, *Rattus norvegicus*, as a representative of another rodent genus closely related to the genus *Mus*. The strains LEW.AVN, ACI, LEW.DA, LEW.1WR2 (all *RT1.B^a*), BUF (*RT1.B^b*), LEW (*RT1.B^l*), BS (*RT1.B^l*), LEW.BN (*RT1.Bⁿ*), LEW.EP, LEW.WP, WF, and LOU (the last three strains are *RT1.B^u*) typed as H-2A.m27-positive and, with one unexplained exception (LEW.BN), RNA isolated from them hybridized with the oligonucleotide probe (Fig. 1). The results suggested that mouse A_β^A forms of the gene are present in these strains. Because the non-deleted form of the gene also exists in the rat¹¹, we conclude that similar polymorphism can be shared not only by different species of the same genus, but also by different genera of the same order.

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1      5      10      15
Arg Asp Ser Pro Arg Asp Phe Val Tyr Gln Phe Gly Gln Cys Tyr Tyr Thr
AGA GAC TCC CCA AGG GAT TTC GTG TAC CAG TTC GAG GGC CAG TGC TAC TAC ACC

20      25      30      35
Asn Gly Thr Gln Arg MET Arg Leu Val Thr Arg His Ile Tyr Asn Arg Glu Glu
ACC GGG ACG CAG CGC ATG CGG CTC GTG ACC AGA CAC ATC TAC AAC CGS GAG GAG

40      45      50
Tyr Val Arg Phe Asp Ser Asp Leu Gly Glu Tyr Arg Ala Leu Thr Glu Leu Gly
TAC GTG CGC TTC GAC AGC GAC CTG GGC GAG TAC CGC GCG CTG ACC GAG CTG GGG

55      60      65      66      67      70
Arg Pro Ser Ala Glu Tyr Trp Asn Lys Gly Tyr Tyr Leu Glu Gln Thr Arg
CGG CCC TCA GCC GAG TAC TGG AAT AAG CAG TAC TAC CTC GAG CAG ACG CGS

75      80      85      90
Ala Glu Leu Asp Arg Val Cys Arg Tyr Asn Tyr Glu Gly Pro Gly Ala Leu Thr
GCC GAG CTG GAC AGG GTC TGC AGA TAC AAC TAC GAG GGG CCG GGG GCT CTC ACC

95      100      105
Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Ala Ile Ser Leu Ser Arg Thr Glu
TCC CTG AGA CGG CTT GAG CAG CCC AAT GTG GCC ATC TCC CTG TCC AGG ACA GAG

110      115      120      125
Ala Leu Asn His His Asn Leu Val Cys Ser Val Thr Asp Phe Tyr Pro Ala
GCC CTT AAC CAC CAC AAC CTG CTG GTC TGC TCA GTG ACA GAT TTC TAC CCA GCC

130      135      140
Gln Ile Lys Val Arg Trp Phe Arg Asn Gly Gln Glu Glu Thr Gly Val Val
CAG ATC AAA GTG CGC TGG TTC CGG AAT GGC CAG GAG GAG ACG ACG GGG GTC GGG

145      150      155      160
Ser Thr Gln Leu Ile Arg Asn Gly Asp Trp Thr Phe Gln Ile Leu Val MET Leu
TCC ACA CAG CTT ATT AGG AAT GGG GAC TGG ACC TTC CAG ATC CTG GTC ATG CTG

165      170      175      180
Glu MET Thr Pro Gln Arg Gly Asp Val Tyr Thr Cys His Val Asp His Pro Ser
GAG ATC ACG CCT CAG CGG GGA GAT GTG TAC ACC TGC CAT GTT GAC CAC CCC AGC

185      190
Leu Glu Ser Pro Val Thr Val Glu Trp
CTT CAG ACC CTT GTC ACA GTG GAG TGG C

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Fig. 2 The nucleotide sequence of the *RT1.B_β*^b gene. Predicted amino-acid sequence is above the nucleotide sequence. Slashes (/) indicate deleted nucleotides.

Methods. The gene was isolated from a complementary DNA library prepared from the BUF (*RT1^b*) strain as described earlier²⁰. The library in the λgt11 vector contained 3 × 10⁷ independent clones. It was screened with an A_β probe and rescreened with the oligonucleotide probe. Fragments of positive clones were subcloned in M13mp18. Single-stranded DNA was purified and sequenced using the chain termination method²¹.

Do the mouse and rat A_β loci really share the same deletion polymorphism? Only one *RT1.B_β* allele has been sequenced¹¹ (the rat *RT1.B_β* locus corresponds to the mouse *H-2A_β* locus) and the sequence does not have deletions at codon positions 65 and 67. On the basis of our serological typing, we have selected one *RT1.B_β* allele that we would predict to have the deletions and sequenced exons 2 and 3 (Fig. 2). The sequence reveals two important features. First, the *RT1.B_β*^b allele of the strain BUF does indeed have the same two deletions in exactly the same positions as the mouse A_β^a alleles do. Second, the exon 2 in which the two deletions occurred is more similar, in terms of nucleotide sequence and predicted amino-acid sequence, to the second exon of the mouse A_β^a (87–88% nucleotide similarity) alleles than it is to the second exon of the mouse A_β^v alleles (81–85% nucleotide similarity) or of the other rat B_β alleles. There is a change in sequence similarity in the third exon, however, where the two rat sequences are more similar to each other than to the mouse sequences.

From these data, we postulate that the A_β^a/A_β^v deletion polymorphism already existed in the last common ancestor of rats and mice. Palaeontologists argue that this ancestral species lived some 10 Myr ago^{12,13}; molecular biologists date the ancestry back much further to some 25 Myr (ref. 14). Because of their common origin, all the A_β^a alleles have remained more closely related to one another than to the A_β^v alleles, regardless of the species bearing them. Somewhere along the rat evolutionary line, intragenic recombination replaced the third exon and the 3' end of one of the B_β^a genes with a segment derived from a B_β^v gene, creating a hybrid gene with the 5' end from a B_β^a and the 3' end from a B_β^v allele.

McConnel and colleagues¹⁵ have recently described two insertions into the non-coding sequence of some of the mouse A_β alleles. These insertions also predate speciation and are fully consistent with our results: all alleles that have one or both insertions belong to the A_β^a group and all those without the insertions belong to the A_β^v group. It will be interesting to see whether the insertions extend as far back as the common ancestor of the rat and the mouse.

There is no reason to believe that this trans-species mode of evolution is limited to deletion polymorphism in rodents: we have evidence that it also applies to nucleotide substitutions at MHC loci in primates^{16,17}. We have established, by sequence

comparisons, that certain human alleles are more similar to certain chimpanzee alleles than they are to other human alleles of MHC loci. It seems, therefore, that transpecies evolution of MHC polymorphism is a rule rather than an exception, as one of us suggested several years ago^{5,6}. The extent to which selection is responsible for this mode of evolution is uncertain as recent analyses indicate that positive selection might act only on the small part of the MHC molecule believed to be involved in antigen binding¹⁸.

An important implication of the trans-species hypothesis of MHC polymorphism is that the initial isolates from which new species begin their evolution must have been fairly large¹⁶. If this were not the case, the polymorphisms would have been lost by random drift in the bottleneck stage. This inference has interesting implications for the theory of speciation in general and for human origins in particular¹⁶.

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HLA-A and B polymorphisms predate the divergence of humans and chimpanzees

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Major histocompatibility complex (MHC) glycoproteins bind processed fragments of proteins and present them to the receptors of T lymphocytes^{1,2}. The extraordinary polymorphism of class I MHC molecules in man (*HLA-A*, *B* and *C*) and mouse (*H-2 K*, *D* and *L*) poses many questions concerning their diversification and evolution. Comparison of allelic sequences within a species suggests diversity is generated by the assortment of point mutations into varied combinations by mechanisms of recombination and gene conversion³⁻⁵. We have now compared class I MHC alleles in two closely related species: humans (*Homo sapiens*) and chimpanzees (*Pan troglodytes*). Chimpanzee homologues of *HLA-A*, *HLA-B* and a non-classical gene have been identified. No features distinguishing human and chimpanzee alleles could be found. Individual *HLA-A* or *B* alleles are more closely related to individual chimpanzee alleles than to other *HLA-A* or *B* alleles. These results show that a considerable proportion of contemporary *HLA-A* and *B* polymorphism existed before divergence of the chimpanzee and human lines^{6,7}. The stability of the polymorphism indicates that hyper-mutational mechanisms are not necessary to account for *HLA-A*, *B* and *C* diversity.

A variable heavy-chain glycoprotein and an invariant light chain called β_2 -microglobulin (β_2 -m, ref. 8) make up the class I MHC molecule. Their association is essential for function and both chimpanzee homologues were analysed. Complementary DNA clones encoding four different class I heavy chains and β_2 -m were isolated. Human⁹ and chimpanzee β_2 -m sequences differ by four silent substitutions in the coding region and seven substitutions in the 3' untranslated region. The two proteins are therefore identical and no differences in the structure, function or polymorphisms of HLA and ChLA molecules can be attributed to β_2 -m.

To assess the divergence among class I heavy-chain sequences we calculated the number of nucleotide differences between pairs of sequences derived from six species: human, mouse, Syrian hamster, miniature swine, rabbit and cow (Fig. 1). Note the chimpanzee sequences are not included. Comparisons between sequences from different species form a different distribution (range, 100–200 substitutions, Fig. 1a) from that obtained by comparisons within a species (range, 1–95 substitutions, Fig. 1b). Thus one can readily distinguish *HLA-A*, *B* and *C* from class I alleles of these distantly related species.

Among *HLA-A*, *B* and *C* sequences, we find that comparisons from different loci form a distribution (range, 45–100 substitutions, Fig. 1c) distinct from that obtained by intra-locus comparisons (range, 1–50 substitutions, Fig. 1d). Thus one can accurately distinguish sequences derived from different loci.

The four chimpanzee heavy chain complementary DNAs (*Ch18*, *Ch25*, *Ch28* and *Ch39*) have typical class I nucleotide sequences⁴ with variability being concentrated in exons two and three, which encode the peptide binding site¹⁰. Pairwise comparisons showed *Ch28* to be exceptionally divergent from *HLA-A*, *B*, *C* and the other chimpanzee sequences (Table 1). Over 100 substitutions were found in all comparisons. In contrast only 14 differences and 98.3% homology were found with the

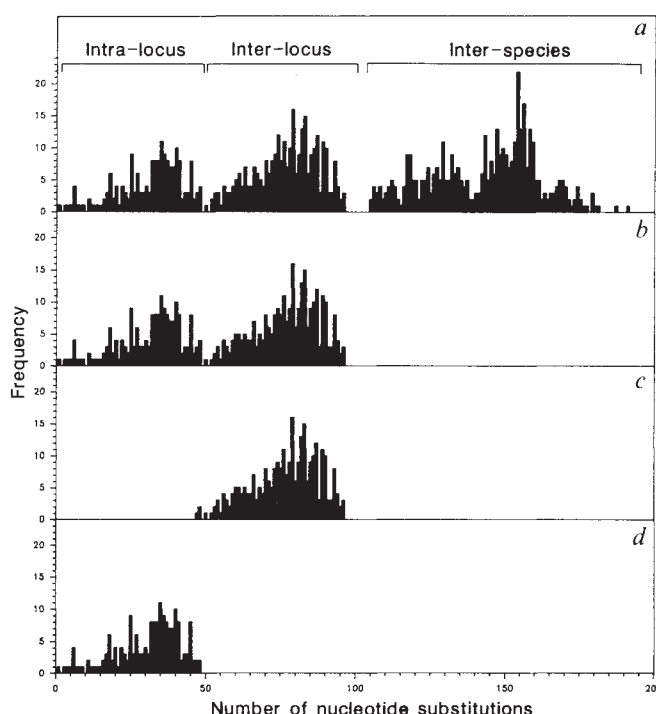


Fig. 1 Frequency distribution of pairwise differences in the sequences of class I MHC genes. *a*, The 822 nucleotides encoding the α_1 , α_2 and α_3 domains of 31 human⁵, six mouse⁴, two cow²³, two miniature swine²⁴, one rabbit²⁵ and one Syrian hamster²⁶ class I genes were compared. Pairwise comparisons were made in all possible combinations and the number of nucleotide differences calculated. A histogram of the frequency of these 903 data points is plotted. *b*, Only the 31 human (*HLA-A*, *B* and *C*) sequences are compared (465 data points) and this distribution is further subdivided into comparisons between alleles of different loci (*c*) and the same locus (*d*). Inter-species comparisons have a range of 100–200 differences and intra-species comparisons have a range of 1–100 differences as indicated by the brackets (*a*). The inter-species distribution is approximately bimodal; one component with pairwise comparisons involving mouse *H-2* sequences (range ~140–200) and a second component involving comparisons between non-murine species (range 100–140). Thus murine sequences have diverged more from those in other species. The distribution of inter-locus differences (*c*) differs from the intra-locus distribution (*d*), showing that genetic exchange between loci is a minor mechanism in the diversification of human class I alleles compared with genetic exchange within a locus. Skewing of the inter-locus distribution to lower values is probably the result of some genetic exchange between the *HLA-A*, *B* and *C* loci. Skewing of the intra-locus distribution to lower values reflects the over-representation of sequences that are related to *HLA-A2* and *HLA-B27*.

non-classical class I *HLA* gene called *5.4* (ref. 11 and D. Geraghty, B. Koller and H. Orr, unpublished results). Thus *Ch28* is the chimpanzee homologue of *5.4*. Non-classical class I genes are poorly conserved in mammalian species¹². The function, if any, of *5.4* and *Ch28* and other non-classical genes is unknown; as is the significance of their conservation during the evolution of the higher primates.

Clones *Ch18*, *Ch25* and *Ch39* have remarkable homology with *HLA-A*, *B*, *C* alleles. The number of substitutions between *ChLA* and *HLA* sequences (Table 1) was between 10 and 86, within the range of 1–96 obtained from comparisons of *HLA-A*, *B* and *C* alleles (Fig. 1). There are only 18 substitutions that have not been found in at least one *HLA-A*, *B*, *C* sequence out of the 152 positions where the chimpanzee sequence differs from the consensus. Moreover there are no substitutions that permit chimpanzee sequences as a group to be distinguished from

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Table 1 Pairwise comparisons of human and chimpanzee class I major histocompatibility complex alleles

Ch25			A11			Ch25			A11		
Allele	Δ	%	Allele	Δ	%	Allele	Δ	%	Allele	Δ	%
A3.2	10	98.8	A3.2	10	98.8	B18	114	86.1	A11	61	92.6
A11	12	98.5	Ch25	12	98.5	Cw3	115	86.0	Cw2.2	64	92.2
A3.1	12	98.5	A3.1	12	98.5	Cw1	115	86.0	Cw1	64	92.2
A1	19	97.7	A1	15	98.2	Bw58	115	86.0	A3.2	65	92.1
Aw68.1	31	96.2	Aw68.1	27	96.7	A32	117	85.8	A3.1	66	92.0
Aw24	33	96.0	Aw24	36	95.6	Bw60	118	85.7	Ch25	68	91.7
A2.1	35	95.7	A32	37	95.5	6.0	118	85.5	Cw3	70	91.5
A32	37	95.5	A2.1	39	95.3	A1	119	85.5	A1	73	91.1
Bw47	68	91.7	Ch39	61	92.6	B44.2	120	85.4	A2.1	80	90.3
Ch39	68	91.7	B27.1	64	92.2	B13	121	85.3	Aw68.1	81	90.2
B27.1	69	91.6	Bw47	66	92.0	Aw68.1	122	85.2	6.0	88	89.3
B40*	72	91.2	B40*	70	91.5	A2.1	124	84.9	E	100	87.8
Bw65	74	91.0	B44.2	73	91.1	Ch18	106	85.4	Ch28	109	86.7
Ch18	65	91.0	Bw65	73	91.1	E	143	82.7	5.4	111	86.4
B18	75	90.9	Bw58	74	91.0	Bw58			Ch18		
B8	76	90.8	B8	74	91.0	Ch39	30	96.4	Bw47	24	96.7
B44.2	76	90.8	B18	75	90.9	B13	32	96.1	B40*	30	95.9
Bw58	80	90.3	Bw60	78	90.5	B44.2	35	95.7	B18	30	95.9
Bw60	80	90.3	Cw1	79	90.4	Bw47	37	95.5	B13	32	95.6
Cw1	80	90.3	Cw2.2	79	90.4	B18	37	95.5	B27.1	33	95.4
6.0	82	90.0	Cw3	79	90.4	B40*	40	95.1	Bw65	33	95.4
B13	83	90.0	B13	81	90.1	B8	44	94.7	Ch39	35	95.2
Cw3	85	89.7	6.0	82	90.0	Bw65	45	94.5	B44.2	35	95.2
Cw2.2	86	89.5	Ch18	73	89.9	Bw60	45	94.5	Bw60	37	94.9
E	103	87.5	E	104	87.3	B27.1	46	94.4	B8	38	94.8
Ch28	114	86.1	Ch28	111	86.5	Ch18	43	94.1	Bw58	43	94.1
5.4	116	85.8	5.4	113	86.3	Cw2.2	59	92.8	Cw1	55	92.4
Ch28			Ch39			Cw1	64	92.2	Cw2.2	60	91.7
5.4	14	98.3	Bw58	30	96.4	Cw3	64	92.2	A3.2	63	91.3
A3.1	108	86.9	B13	35	95.7	Aw24	66	92.0	A3.1	64	91.2
Ch39	109	86.7	Bw47	38	95.4	A32	68	91.7	Cw3	65	91.0
A3.2	111	86.5	Ch18	35	95.2	A11	74	91.0	Ch25	65	91.0
A11	111	86.5	B27.1	43	94.8	A3.2	75	90.9	Aw24	66	90.9
Bw47	111	86.5	Bw65	44	94.7	A3.1	76	90.7	A32	67	90.8
Cw2.2	111	86.5	B18	44	94.7	A1	79	90.4	A11	67	90.8
B40*	112	86.4	B44.2	44	94.7	Ch25	80	90.3	A1	74	89.8
Aw24	113	86.3	B40*	44	94.7	A2.1	82	90.0	A2.1	78	89.2
Bw65	113	86.3	B8	48	94.2	Aw68.1	87	89.4	Aw68.1	79	89.1
B27.1	113	86.3	Bw60	49	94.0	6.0	88	89.3	6.0	87	88.0
B8	113	86.3	Aw24	56	93.2	E	105	87.2	E	101	86.1
Ch25	114	86.1	A32	60	92.7	Ch28	115	86.0	5.4	103	85.8
						5.4	116	85.9	Ch28	106	85.4

The four chimpanzee class I sequences (*Ch18*, *Ch25*, *Ch28* and *Ch39*), *HLA-A11* and *HLA-Bw58* are compared to each other, to 19 *HLA-A*, *B*, *C* genes and to 3 expressed, non-classical *HLA* genes (*HLA-E*, *5.4* and *6.0* refs 21, 22 and D. Geraghty, B. Koller and H. Orr, unpublished data). The number of nucleotide substitutions (Δ) and the percentage similarity (%) are shown. These comparisons show that *HLA-A11* and *HLA-A3* are similar to *Ch25*, *HLA-Bw58* is similar to *Ch39* and *HLA-Bw47* is similar to *Ch18*. For *Ch25*, *Ch28* and *Ch39* and all *HLA* sequences the percentage similarity is calculated from a total of 822 base pairs that comprise exons two, three and four. *Ch18* is a truncated clone starting at position 99 in exon two and so comparisons involving *Ch18* were made over 724 bases only. For this reason the % similarity calculated for a given value of Δ is different for *Ch18* and the other three clones.

human sequences. This property extends to the predicted amino-acid sequences of the *ChLA-A*, *B* molecules. Of 87 residues where the three *ChLA-A*, *B* sequences differ from the *HLA-A*, *B*, *C*-derived consensus only 13 are not found in the human sequences (Fig. 2).

There is, however, unequivocal evidence for locus specificity, in contrast to this lack of species specificity (Table 1). For example *Ch25* is more closely related to all *HLA-A* alleles than it is to either *HLA-B* or *HLA-C* alleles and *Ch18* and *Ch39* are more closely related to all *HLA-B* alleles than to *HLA-A* or *C* alleles. Despite both the rudimentary nature of our understanding of the chimpanzee MHC¹³ and the lack of characterization of the products of these cDNAs, it is almost certain that they represent the classical class I MHC molecules of the chimpanzee. Thus *Ch25* can be said to be the product of the *ChLA-A* locus whereas *Ch18* and *Ch39* are products of the *ChLA-B* locus.

Certain *HLA* alleles are more closely related to the chimpanzee alleles than they are to other *HLA* alleles. This is most marked for *HLA-A1*, *A3.1*, *A3.2*, *A11* and *Ch25* (Table 1 and Fig. 3). The similarity of their sequences indicates that they diverged from an ancestral sequence that existed before separation of the human and chimpanzee lines. The pattern of substitutions within this group is analogous to that of other families of class I alleles such as that involving *HLA-A2* and *HLA-A28*¹⁴. Of the 26 variable positions in exons two and three, 20 involve coding substitutions and 17 of these change residues that may directly affect binding site interactions with peptides and T-cell receptors¹⁰. It is therefore likely that many of the differences between *HLA-A1*, *A3.1*, *A3.2*, *A11* and *Ch25* will affect T-cell responses, as has already been shown for residue 152 (ref. 15), and that these alleles have been fixed as a result of positive selection.

HLA-B alleles show a greater diversity than *HLA-A*, and the

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Fig. 2 Comparison of the structure of chimpanzee and human class I MHC heavy-chain glycoproteins. The consensus sequence is derived from 39 HLA-A, B and C sequences⁵. Dashes indicate identity with this sequence. Substitutions in the chimpanzee sequences (*Ch18*, *Ch25*, *Ch39*) not found in the HLA sequences are given in bold. ▲ Mark positions in HLA-A, B, C where locus-specific residues are found. *Ch25* has A locus residues and *Ch18* has B locus residues at these positions. *Ch39* has B locus residues with the exception of position 189 where it has methionine in common with HLA-A molecules. Δ Mark positions in which the classical HLA-A, B and C and ChLA-A and B products are identical and the non-classical *Ch28* molecule is different. *Ch28* contains no sequence from exon seven (indicated by*) suggesting a different pattern of messenger RNA splicing for this gene. The critical residues involved in N-glycosylation (N86) and the formation of disulphide bonds (C101, C164, C203 and C259) are conserved in the four chimpanzee sequences. One gap in the transmembrane domain is introduced to maximize homology. The chimpanzee B-cell line 'Tank' was obtained by transformation with Epstein Barr virus of peripheral blood lymphocytes from a recently born male. The 'Tank' cells show reactivity with human alloantisera specific for the HLA-A10 and A11 cross-reacting groups and for the HLA-Bw4 epitope. The first reactivity is attributed to the *Ch25* molecule which is closely related to HLA-A11 and the second reactivity to the *Ch39* molecule which has a characteristic Bw4 sequence, RIALR, at residues 79–83 (ref. 27).

Methods. Complementary DNA clones from 'Tank' cells were made by the procedure of Gubler and Hoffman²⁸. λgt10 clones, hybridizing to the class I heavy chain (*B7* cDNA)²⁹ or β₂-m probes⁹, were plaque purified and the *Eco*RI inserts subcloned into M13mp18. Sequencing of both strands with the dideoxy termination method used M13 sequencing primers and a series of oligonucleotides specific for conserved regions within the coding sequence²⁰.

Fig. 3 Positions of variation between *HLA-A1*, *A11*, *A3.1*, *A3.2* and *Ch25*. Substitutions between the coding regions of *HLA-A1* (ref. 5), *A3.1* (ref. 30), *A3.2* (ref. 31), *A11* (ref. 32) and *Ch25* genes and a consensus derived from these sequences is shown. Coding substitutions are given in uppercase and silent substitutions in lowercase. The numbering of the nucleotides starts with the initiation codon. The amino acid residue in the mature protein that is affected by the individual nucleotide substitutions is given in parentheses.

Positions in the protein with high variability⁵ are indicated by *, those positions that line the peptide combining site and are thought to interact directly with bound peptide, or the T-cell receptor are indicated by ‡. The number of mismatches between each gene and the consensus over the total positions compared is given at the right. * indicate exons in which sequences have not been determined.

	Consensus	ATGC	TAGGAGTGTGA	GTACCCACGCACGTA	TG	TC	T	
Ch25	--Ct	--tG--CC--	-----	-----	ca	Ct	*	10/34
A11	****	Ac-----	-C-----	-CG--g	--	--	--	6/31
A3.2	****	-----C	--g-T--T--	-----	--	**	*	4/28
A3.1	CA--	-----C	--g-ATT-T--	-----	--	--	C	9/35
A1	----	--A--A-CCA-	cC-T--GT-gCGCG-	----	--	--	--	15/35

(codon/amino acid)
nucleotide #

same may be true for *ChLA-B* and *ChLA-A*. *Ch39* is related to *HLA-Bw58* and *Ch18* to *HLA-Bw47*. However, the differences are greater than between *Ch25* and its related *HLA-A* alleles (Table 1).

All four chimpanzee class I alleles examined are related to alleles of particular *HLA* class I loci. Most substitutions (88%) at positions of variability in the chimpanzee sequences are shared with *HLA* alleles and one cannot *a priori* distinguish between alleles derived from the two species. The existence of *HLA-A* or *B* alleles which are more closely related to particular *ChLA-A* or *B* alleles than they are to other *HLA-A*, *B* alleles is particularly important. As the chimpanzee used in this study was randomly chosen it is likely that these properties will extend to other *ChLA* alleles.

The similarity between individual *HLA* and *ChLA* sequences, both in the nature of the substitutions and their combination, clearly indicates that much of the contemporary diversity in *HLA* and *ChLA* class I alleles had accumulated before divergence of the two species 3.7–7.7 million years ago^{6,7}. This pattern of MHC polymorphism has been said to result from trans-species evolution¹⁶.

These conclusions from primate class I MHC molecules are similar to those obtained from peptide mapping of class I molecules and restriction site mapping of class II MHC alleles in mice^{17,18}. These results, combined with estimates of nucleotide substitution^{19,20} and the failure to find newly arisen *HLA* types in family studies, collectively argue against the involvement of special hypermutational mechanisms in the generation of class I MHC diversity.

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Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice

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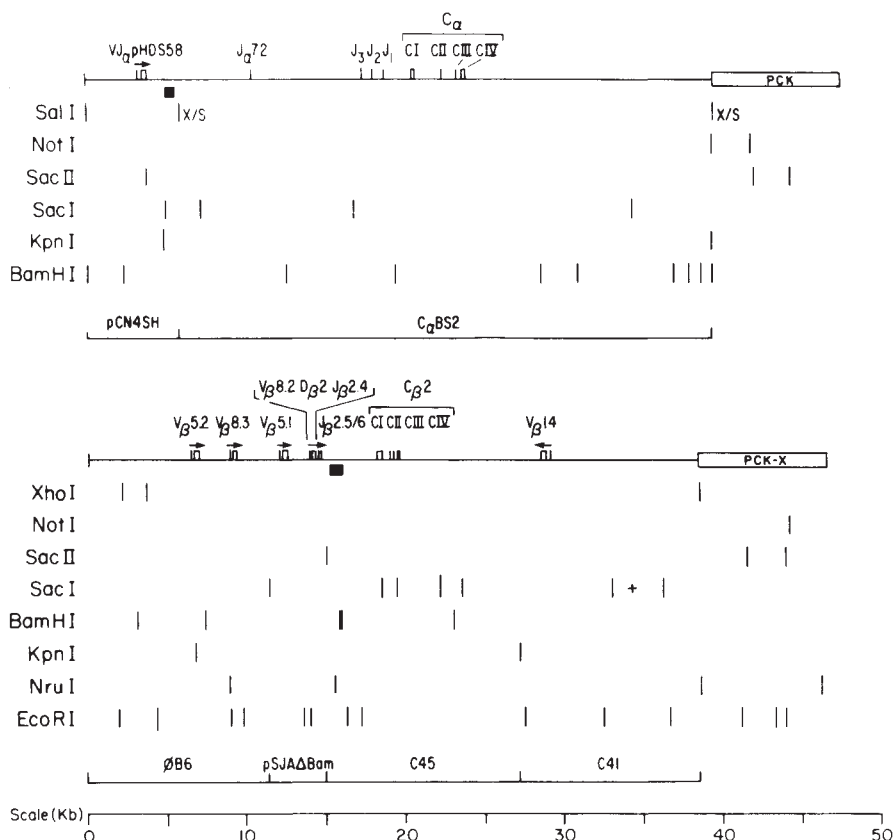
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The major problem in the study of T-cell development is that of tracking thymocytes of a given specificity. Recent studies^{1–4} have exploited natural correlations between the expression of a particular *V β* gene segment and T-cell receptor (TCR) specificity. We and others (refs 5, 6 and M. Davis, personal communication) have taken an alternative approach. We have generated transgenic mice expressing the $\alpha\beta$ antigen receptor from the cytotoxic T-lymphocyte clone 2C (ref. 7). In transgenic mice of the same haplotype as the 2C clone, the 2C TCR was expressed on 20–95% of peripheral T cells. Very few of these T cells carried the CD4 antigen; the vast majority were CD4[–]CD8⁺ and were able to lyse targets with the same specificity as the original 2C clone. These results indicate that the $\alpha\beta$ heterodimer transfers specificity to recipient cells as expected from earlier studies^{8–11}, and that receptor specificity in T-cell repertoire selection is determined by both $\alpha\beta$ heterodimer and CD4 or CD8 accessory molecules.

The cytotoxic T-cell clone 2C was derived from BALB.B (H-2^b) mice, has specificity for the L^d class I major histocompatibility complex (MHC) antigen¹² and its TCR can be identified by two monoclonal antibodies, F23.1¹³ and 1B2¹². F23.1 binds to antigenic determinants on all three members of *V β* 8 family¹⁴. 1B2 is a clonotypic antibody specific for the 2C TCR¹⁵. Using

Fig. 1 2C α - and β -chain constructions of functionally-rearranged genes. Cosmids were used because initial transgenic experiments by our group and others³ failed to express β -transgenes when a few kb of flanking sequence were used. Mice were screened for integration of transgenes using a 650-base-pair (bp) *Bam*HI-*Cla*I $J_{\beta}2$ probe and a 550-bp *Eco*RI-*Sac*I J_{α} probe shown as dark boxes below each construction.

Methods. Two overlapping cosmids were isolated from a DBA/2 liver cosmid library¹⁹, and screened with a C_{α} complementary DNA probe. A 33.5-kb cosmid fragment from C_{α} DBA2 containing approximately 14-kb 5' and 3' of the C_{α} exons was isolated using the *Sal*I site in the vector pTCF²⁰ and the natural *Kpn*I site 3' of the C_{α} gene². This insert was subcloned into the *Sal*I site of pCK (pTCF modified to include *Not*I and *Sfi*I sites at the *Cla*I site) using a trimolecular ligation with a *Kpn*I-*Xho*I adaptor phosphorylated at the *Xho*I end. This ligation generated an intermediate, C_{α} BS2, capable of accepting a rearranged VJ_{α} gene segment of up to 9 kb into the unique *Sal*I site 5' of the C_{α} . Using a V_{α} probe from the cDNA pHDS58⁷, 18 phages were isolated from a Lambda Dash (Stratagene, LaJolla, California) library constructed from 2C DNA. Six of these phages hybridized to both a J_{α} pHDS58 and a VJ_{α} junction-specific oligonucleotide. A 5.85-kb fragment was isolated from one of these phages, ϕ CN4, using a *Sal*I site in Lambda Dash 3-kb 5' of the V_{α} gene and a natural *Hind*III site 2-kb 3' of the J_{α} gene segment. This fragment was subcloned into a *Sal*I-*Hind*III Bluescribe vector (Stratagene) and a *Xho*I linker was introduced into the Klenow-blunted *Hind*III site to obtain pCN4SH. The rearranged VJ_{α} gene was shown to be identical to the cDNA pHDS58 by dideoxynucleotide sequencing. The 2C VJ_{α} gene was excised from pCN4SH as a *Sal*I-*Xho*I insert, ligated into the *Sal*I site of C_{α} BS2, and screened for proper orientation to complete the 2C α -chain construct. The 2C β -chain gene was constructed by addition of flanking segments from several phage and cosmid clones to a 5-kb *Sac*I-*Bam*HI Bluescribe subclone of the functionally-rearranged 2C VDJ_{β} gene segment, pSJA Δ Bam. The nucleotide sequence of the coding region of this genomic subclone was identical to the sequence of the 2C β -chain cDNA, 2C1 β 2no.18. The 5-kb genomic subclone was excised from pSJA Δ Bam as a *Sac*I-*Sal*I insert and re-subcloned into a *Sac*I-*Xho*I Bluescribe RI-X vector (Bluescribe modified by *Xho*I linker insertion into the *Eco*RI site). A *Sal*I-*Sac*I fragment was made from this plasmid and 11 kb of 5' flanking sequence was added by ligating in a *Sal*I-*Sac*I fragment from ϕ B6 containing the $V_{\beta}5.2$ and 8.3 genes²². A 14-kb *Sac*II-*Sal*I insert, phosphatase-treated at the *Sac*II site, was excised from this intermediate and ligated in a trimolecular reaction with a *Xho*I phosphatase-treated pCK-X vector (pCK modified by replacement of the *Sal*I sites with a *Xho*I site) and a 25-kb *Sac*I-*Xho*I insert excised from the cosmid C41/45. C41/45 was created by splicing together two BALB/c cosmids²³, C41 and C45, at the *Kpn*I site between $V_{\beta}14$ and $C_{\beta}2$ (ref. 24).



strains of mice lacking the $V_{\beta}8$ gene family¹⁶, α and β constructions linearized with *Not*I (Fig. 1) were co-injected into fertilized F₂ eggs obtained from mating (C57L $\times$ SJL)F₁ mice. Three transgenic founder mice, P, H and D, were produced. Fifty mice, P.1 to P.50, were derived by mating founder P, a b $\times$ b haplotype animal, to (C57L $\times$ SJL)F₁ females. Transgenic offspring segregated into two types. Ten (type B) possessed approximately eight copies of the β and one copy of the α -transgene. Seventeen offspring (type A) possessed two copies of the β - and four copies of the α -transgene.

Northern blots of total thymus RNA from transgenic and non-transgenic littermates were hybridized with V_{α} pHDS58 and $V_{\beta}8$ probes. Full-length transcripts of 1.7 and 1.3 kilobases (kb), respectively, were evident in RNA from transgenic animals only. Using RNase protection analysis (Fig. 2), the transcription of transgenes was shown to be strongest in the lymphoid tissues (thymus and spleen); little or no transcription was found in the non-lymphoid tissues (brain, heart, kidney and liver).

Surface transgene expression in eight offspring of founder P was analysed by two-colour flow cytometry. In these mice, almost all single positive CD4⁺ and CD8⁺ spleen cells were F23.1⁺, indicating that almost every T cell expressed the 2C β -chain. In two-colour analysis of F23.1 versus 1B2, the percentage of F23.1⁺1B2⁻ cells was variable. The percentage of 1B2⁺

cells ranged from 20 to 95% of F23.1⁺ cells. This variation was reflected in both spleen and thymus cells. In animals with high clonotype expression, the CD4/CD8 ratio of total T cells was clearly skewed towards CD8⁺ cells compared with non-transgenic littermates where the CD4/CD8 ratio of T cells in the periphery was 1.5 ± 0.1 ($n = 3$). The skewing was caused solely by selective expression of the 2C TCR on CD8⁺ T cells. In an animal with lower clonotype expression (Fig. 3), 62% of immunoglobulin-negative spleen cells were 1B2⁺CD8⁺ and only 1.1% were 1B2⁺CD4⁺. In contrast, 17% of immunoglobulin-negative spleen cells were 1B2⁻CD4⁺ and 7.7% were 1B2⁻CD8⁺. In the other mice analysed, the lowest skewing of the CD4/CD8 ratio of 1B2⁺ T cells was 0.1; mouse P.24 contained 4.6% 1B2⁺CD4⁺ and 47% 1B2⁺CD8⁺ spleen cells. In contrast, the CD4/CD8 ratio of 1B2⁻ T cells ranged from 1 to 2.5. These CD4/CD8 ratios in F23.1⁺ clonotype-negative T cells are consistent with data reported by Uematsu *et al.*⁵, and indicate that surface expression of the β -chain transgene alone does not cause skewing of the CD4/CD8 ratio.

Thymocytes were examined for surface transgene expression to investigate the skewing found in the periphery. In mouse P.19, 74% of thymocytes were CD4⁺CD8⁺ and 75% were 1B2⁺, indicating that a large fraction of double positive thymocytes expressed the 2C TCR. In mouse P.24, 80% of thymocytes were

1B2⁺. Selective expression of clonotype on CD8⁺ splenocytes was reflected in an increased population of CD4⁺CD8⁺ thymocytes (Fig. 1f), relative to littermate controls where 3–5% of thymocytes were CD4⁺CD8⁺ and 10–15% were CD4⁺CD8[−]. The population of 1B2⁺CD4[−] thymocytes was always larger than that of 1B2⁺CD8[−] thymocytes, which is consistent with clonotype expression being the cause of the selective increase in CD4⁺CD8⁺ thymocytes.

The selective expression of 1B2 on CD8⁺ T cells predicts that such cells should have lytic activity similar to that of the original clone 2C. Freshly isolated splenocytes from a transgenic animal with 95% 1B2⁺ T cells had no lytic activity against L^d-bearing targets. However, after polyclonal activation by the T-cell mitogen concanavalin A (Con A), in the presence of conditioned media, spleen cells from transgenic mice, but not from littermate controls, were stimulated to lyse L^d-bearing tumour cells (P815) but not other non-L^d-bearing tumour cells (BW5147 or EL4). The ability of transgenic T cells to respond to MHC products was also tested by using irradiated spleen cells from BALB/c (H-2^d) and dm2 mice, a BALB/c L^d deletion mutant¹⁷, as stimulators.

In a three-day proliferation assay using recombinant IL-2, spleen cells from mouse P.19 responded to purified T-cell stimulators from BALB/c mice (1170±30, ³H-thymidine incorporation, c.p.m. × 10^{−2}) but not dm2 mice (43±3) when compared to the incorporation with medium plus IL-2 alone (41±30) or the lectin Con A plus IL-2 (1380±80). A control littermate responded weakly to BALB/c (33±4) when compared to medium plus IL-2 alone (17±1). Transgenic T cells incubated for five days with BALB/c stimulators were able to lyse BALB/c, but not dm2 lymphoblast targets. Lysis of BALB/c targets was blocked by addition of 1B2. The ability of activated 1B2⁺ cells to mediate lysis was confirmed by covalently attaching 1B2 to a non-L^d bearing tumour cell and demonstrating antibody-dependent lysis (Table 1). Lysis dependent on 1B2 expression

Table 1 Lytic specificity of T cells from transgenic and control mice after polyclonal activation

Responder	E:T	% Specific ⁵¹ Cr release		
		BW5147	1B2-BW5147	P815
Type A P.40	2:1	2	63	67
	10:1	7	68	66
Control P.42	2:1	2	3	0
	10:1	3	5	1
2C	2:1	1	40	55
	10:1	3	66	60

Mouse P.40, an eight-week-old b × b-haplotype female littermate of P.42, had 85% 1B2⁺ T cells. Splenocytes from both animals were stimulated for three days in the presence of 4 μg ml^{−1} Con A, 10% rat Con A supernatant, and 10% supernatant from alloreactive mixed-lymphocyte cultures (BALB/c anti-BALB.B). Cells were then washed and assayed for lytic activity along with the 2C clone in duplicate at two effector:target ratios (E:T) against 2 × 10⁴ labelled targets in a 4 h ⁵¹Cr release assay. 1B2 was attached to target cells as described previously¹⁵.

by T cells from mouse P.40 was even more efficient than lysis of the same target cells by the 2C clone. Thus, it is likely that most, if not all, of the 1B2⁺ T cells expressed functional receptor, and that these cells were cytolytic.

The most striking feature of the transgenic mice was the surprisingly small percentage of 1B2⁺ cells in the periphery which expressed the CD4 accessory molecule. It is unlikely that the skewing towards expression of a TCR from an alloreactive T lymphocyte on CD8⁺ cells is a result of gross disruption of thymocyte development by the introduction of functionally-

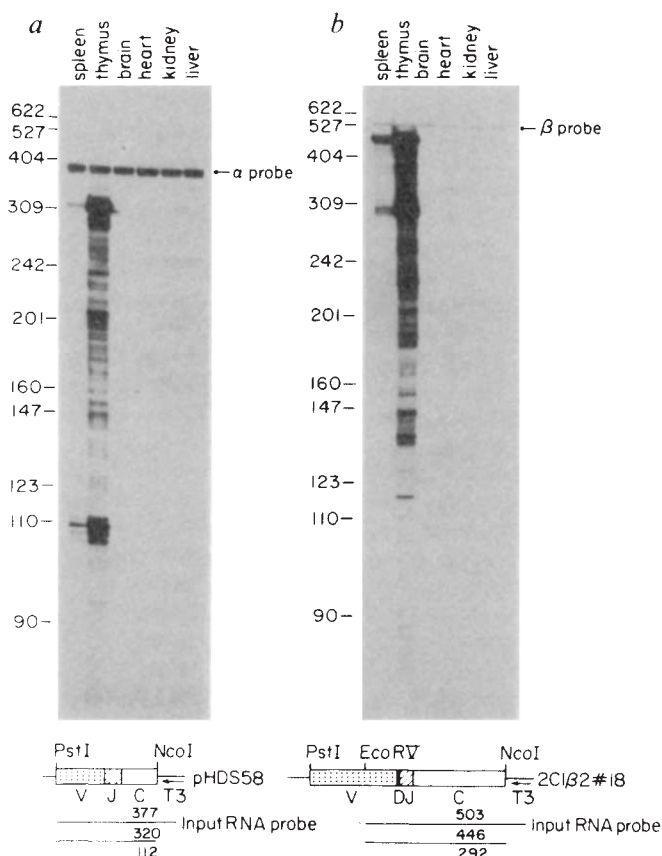


Fig. 2 Tissue specificity of transgene transcription. RNase protection analysis of a seven-week-old type B, bxs-haplotype female, P.27. Bands corresponding to protected α - and β -transgene transcripts were not present in parallel experiments on non-transgenic littermates.

Methods. DNA templates for uniformly-labelled antisense RNA probes were prepared by subcloning into Bluescript vectors (Stratagene) fragments from the 2C cell line cDNAs, pHDS58 and 2C1 β 2no.18. Both α - and β -probes were hybridized against ~30 μg of RNA from six tissues prepared by the guanidinium isothiocyanate method. Probes were prepared according to Stratagene protocols and RNase protection carried out according to the method of Melton *et al.*²⁵.

rearranged receptor genes. Firstly, CD4⁺CD8⁺ thymocytes express the 2C TCR. If double positive cells are the precursors of mature, single positive thymocytes, as several groups have suggested^{4,6,18}, thymocytes expressing the 2C TCR would seem to have the potential to become either CD4⁺ or CD8⁺ T cells. Secondly, the skewing is clonotype specific. Expression of the β -transgene alone does not cause skewing towards CD8⁺ cells, whereas co-expression of α - and β -transgenes does, strongly suggesting that the specificity of the receptor is determining the skewing. Our data demonstrate that repertoire selection in the thymus involves selection of T-cell specificity which is determined by both $\alpha\beta$ heterodimer and CD4 or CD8 accessory molecules. In a b-haplotype thymus, either there is negative selection against the 2C receptor with the CD4 molecule or the 2C receptor can only undergo positive selection to mature with the CD8 molecule. Although our data do not allow us to distinguish between these two possibilities, several groups report that tolerance induction seems to occur before the divergence of CD4⁺ and CD8⁺ T-cell lineages^{4,6}, indicating that negative selection of TCR in association with either CD4 or CD8 should result in complete elimination of the clonotype.

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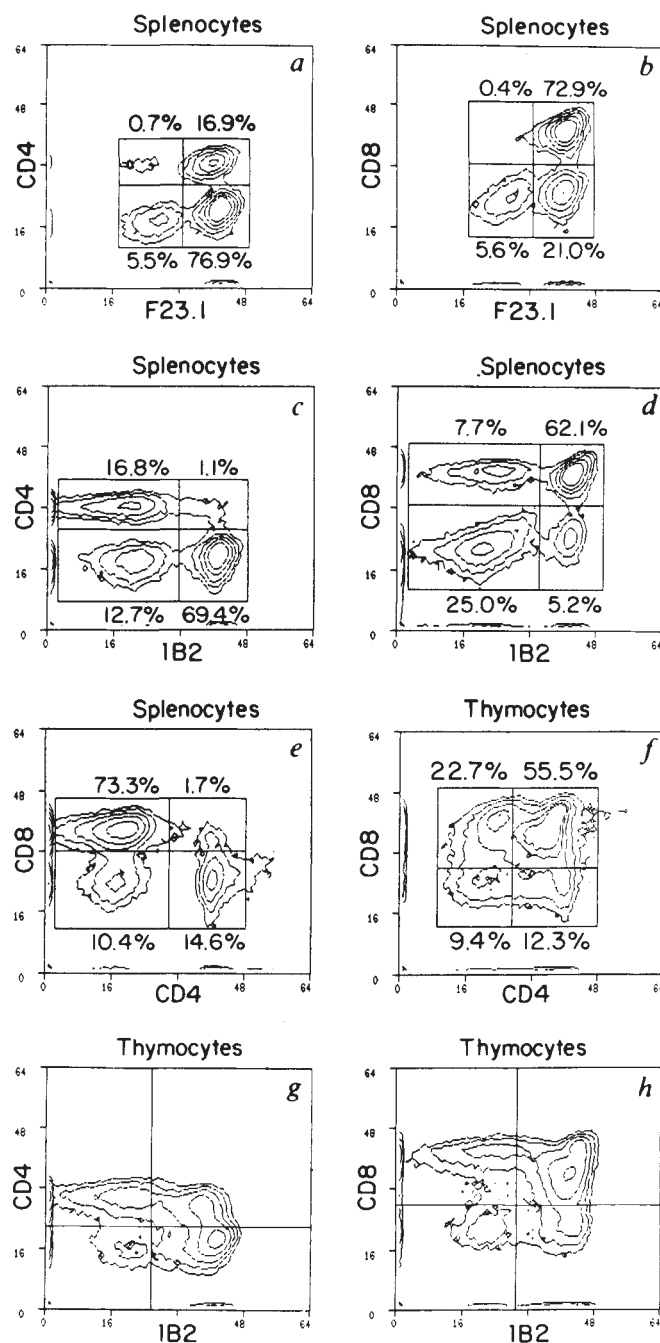


Fig. 3 Two-colour flow cytometric analysis of transgene surface expression in 14-week-old type B, bxb-haplotype females. Immunoglobulin-negative splenocytes were isolated from P.19, thymocytes from P.24. Thymocytes from three non-transgenic littermates did not stain positively with either F23.1 or 1B2. Approximately 5–6% of immunoglobulin-negative splenocytes from these control animals did stain diffusely with F23.1 and 1B2. We attribute this staining to nonspecific interactions with immunoglobulin-positive contaminating cells because approximately 5–7% of immunoglobulin-negative splenocytes from transgenic and non-transgenic mice stained positively with anti-mouse immunoglobulin reagents.

Methods. Red blood cells were removed from cell populations by ammonium chloride lysis, and splenocytes were treated further by passage over a T-cell recovery column (Sci Can Diagnostics, Edmonton) to remove immunoglobulin-positive cells. First-step reagents used were biotinylated 1B2, biotinylated F23.1, 53–6.72 (rat anti-mouse CD8)²⁶ and RL172.4 (rat anti-mouse CD4)²⁷. For the CD4×CD8 double, GK1.5 (rat anti-mouse CD4)²⁸ and AD4(15) (mouse anti-mouse CD8, Cedarlane, New York) were used. Second-step reagents used were streptavidin-phycoerythrin (PE) conjugate (Chromoprobe, California) and fluorescein isothiocyanate (FITC)-conjugated goat anti-rat immunoglobulin cross-absorbed against mouse immunoglobulin (Southern Biotechnology Associates, Alabama). For the CD4×CD8 double, cross-absorbed, FITC-conjugated, goat anti-rat IgG and PE-conjugated goat anti-mouse IgM (Southern Biotechnology) were used. 100,000 FACS events were analysed for each contour plot using a Becton-Dickinson FACS 440. In these plots, PE and FITC fluorescence are plotted on the x and y axes, respectively. Contour intervals used were 10, 30, 90, 270 and 810 cells (an additional contour at 180 was added in f). In the thymocyte analyses, lines used to define positive and negative cells were set against the outermost contour of staining controls in which first-step reagents were omitted. In the immunoglobulin-negative splenocyte analyses, distinct cell populations were boxed and the percentage of total cells calculated. The boxes containing negative cell populations were extended beyond second-step control staining boundaries to include the majority of nonspecifically staining cells.

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Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo

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The formation of the unsegmented terminal regions of the *Drosophila* larva, acron and telson requires the function of at least five maternal genes (terminal genes class¹). In their absence, the telson and acron are not formed. One of them, *torso* (*tor*)², has gain-of-function alleles which have an opposite phenotype to the lack-of-function (*tor*⁻) alleles: the segmented regions of the larval body, thorax and abdomen, are missing, whereas the acron is not affected and the telson is enlarged. In strong gain-of-function mutants, the pair-rule gene *fushi tarazu*³ (*ftz*) is not expressed, demonstrating the suppression of the segmentation process in an early stage of development. The *tor* gain-of-function effect is neutralized, and segmentation is restored in double mutants with the zygotic gene *tailless*^{4,5} (*tll*), which has a phenotype similar (but not identical) to that of *tor*⁻. This suggests that *tor* acts through *tll*, and that in the gain-of-function alleles of *tor*, the *tll* gene product is ectopically expressed at middle positions of the embryo, where it inhibits the expression of segmentation genes like *ftz*.

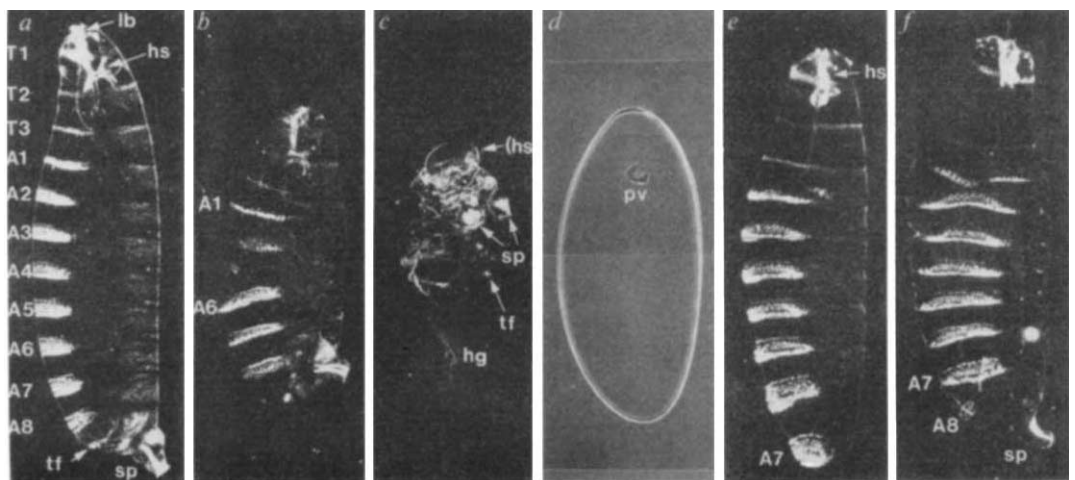
In eggs derived from flies homozygous for null alleles of the *tor* locus, all embryos fail to develop the most anterior and the most posterior regions of the larva (Fig. 1e). In the head, the labrum is not formed and the head skeleton is reduced in size. At the posterior, all elements posterior to the seventh abdominal segment are deleted, including the eighth abdominal segment and the telson which includes spiracles, anal region, and the

posteriorly-derived portions of the gut⁶ (hindgut and posterior midgut). This *tor* lack-of-function phenotype is indistinguishable from that of mutations in four other maternal genes in *Drosophila*: *trunk*², *torso-like* (H.-G. Frohnhofer *et al.*, unpublished), *fs(1)Nasrath*⁷ and *fs(1)polehole*⁸ (the terminal genes class).

Unlike the other genes, there are *torso* gain-of-function alleles which result in a strikingly different phenotype. This gain-of-function phenotype is complementary to the lack-of-function phenotype: elements of the anterior and posterior ends of the embryo are formed, but the large segmented region of thorax and abdomen display defects in development (Fig. 1b-d). Three ethylmethane sulphonate (EMS)-induced mutations have been isolated which are characterized as gain-of-function alleles, because their effect on thorax and abdomen can be reverted by further mutagenesis. Two of them are dominant (*tor*^{Y9} and *tor*⁴⁰²¹), whereas *tor*^{RL3} may be described as a recessive gain-of-function mutant, as one copy of the mutant allele (*tor*^{RL3}/*tor*⁺, *tor*^{RL3}/*tor*⁻) does not affect development (*tor*^{RL3} was isolated by T. Schüpbach and called *spliced*). Revertants of all three alleles have been recovered in screens where flies were selected for regained fertility (see legend to Fig. 1). The revertants display *tor* lack-of-function phenotypes and fail to complement alleles of *tor* (a more detailed genetic analysis will be published elsewhere).

Because the three gain-of-function alleles differ in the strength of their effect on segmentation (*tor*^{RL3} < *tor*^{Y9} < *tor*⁴⁰²¹), a phenotypic series from wild-type (Fig. 1a) to strong gain-of-function phenotypes (Fig. 1d) can be established. In weakly mutant embryos, head and telson are normal, and pattern elements of thorax and anterior abdomen are deleted to a variable extent (Fig. 1b). With increasing strength of phenotype, all head, thoracic, and abdominal segments are gradually lost (Fig. 1c), until finally, in the strongest gain-of-function situation, no cuticle is formed (Fig. 1d). These embryos do differentiate, but they only form tissues of those internal organs that are derived from the terminal anlagen of the egg, for instance posterior midgut and Malpighian tubules.

Fig. 1 The phenotype of *torso* lack- and gain-of-function alleles. Dark-field and phase-contrast photographs of cuticular preparations. *a*, Wild-type. The denticle belts mark the thoracic (T1 to T3) and abdominal segments (A1 to A8). Whereas most of the involuted head skeleton (hs) derives from segmental anlagen, the labrum (lb) is thought to be part of the asegmental acron¹. The telson includes the anal pads, the tuft (tf), and the spiracles (sp) with the prominent filzkörper. For detailed description of the terminal cuticular structures see refs 6 and 20. *b-d*, *torso* gain-of-function phenotypes. The embryo in *b* is an example of a weak phenotype (maternal genotype *tor*^{RL3}/*tor*^{RL3}):



head (including labrum) and telson are unaffected, whereas the segments A2-A4 are partially or completely deleted. In intermediate phenotypes (*c*, *tor*^{Y9}/*tor*⁺), all derivatives of the segmental anlagen of head, thorax and abdomen are defective. Note the enlarged telson with tuft, malformed spiracles, and a hindgut (hg) which failed to involute. In strong phenotypes (*d*, *tor*⁴⁰²¹/*tor*⁺), remainders of the proventriculus (pv) are sometimes found, otherwise no cuticle is formed (in this embryo the vitelline membrane has not been removed). Tissues of internal organs like posterior midgut or Malpighian tubules still develop (Fig. 2). *e*, *torso* lack-of-function phenotype, maternal genotype *tor*^{YRE16}, *tor*^{YRE16} is a revertant of *tor*^{Y9}. Its phenotype is indistinguishable from that of the *tor* alleles previously described². Labrum, A8 and telson are missing, and the head skeleton is collapsed. The thoracic and most of the abdominal segments are not affected. *f*, *tor* lack-of-function embryo (*tor*^{PM}/*tor*^{PHI}), injected with wild-type cytoplasm. The cytoplasm was derived from 50% egg length in the donor, and placed at the posterior pole of the recipient embryo. A spiracle is formed that is never seen in control embryos (see Table 1).

Methods. To show that the gain-of-function mutations were indeed *torso* alleles, we performed reversion experiments based on phenotypic rescue. We took advantage of the extraordinary complementation behaviour of *tor*^{RL3}: mutagenized males were crossed with appropriate females, so that in the test generation flies of the genotypes *tor*⁴⁰²¹/*tor*⁺, *tor*^{RL3}/*tor*^{RL3} and *tor*^{Y9}/*tor*^{RL3} were obtained that were sterile as a result of the *torso* gain-of-function effect (the mutagenized chromosome is marked by an asterisk). Because *tor*⁻/*tor*⁺ and *tor*⁻/*tor*^{RL3} are fertile, the reverted alleles were selected by the occurrence of fertile females. Revertants were obtained at a rate of about one in 500 tested chromosomes, using EMS as mutagen. As *tor*^{RL3} is temperature sensitive, the experiments were performed at 27 °C.

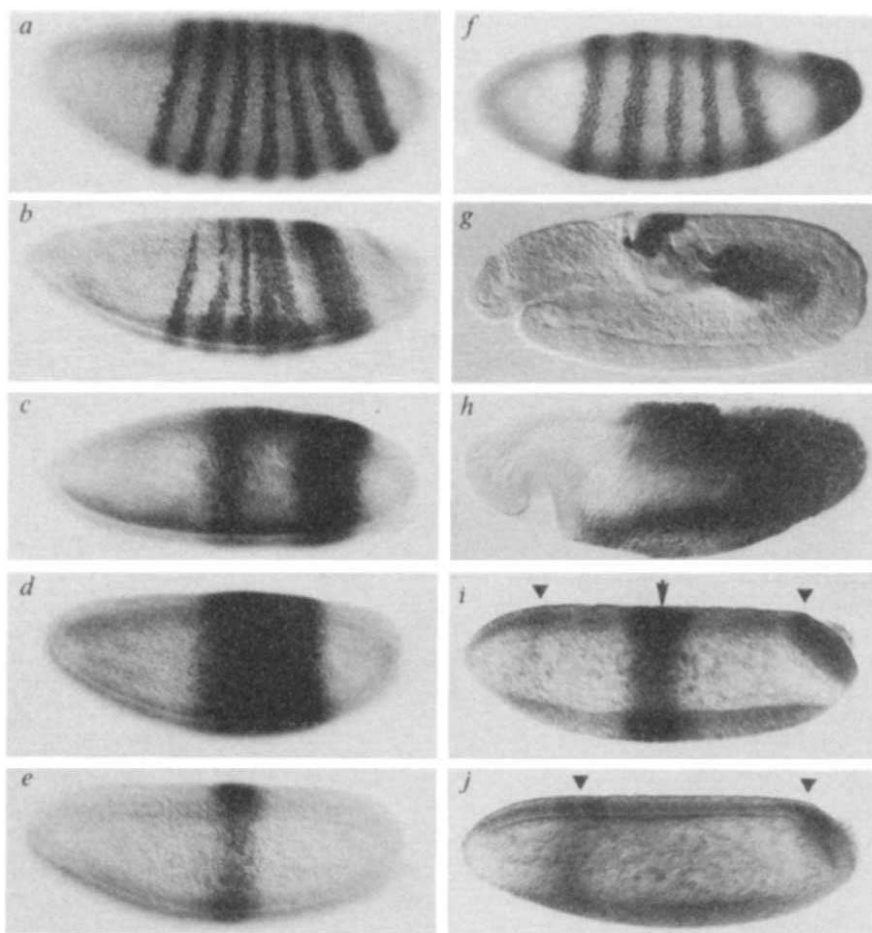


Fig. 2 The effect of *torso* mutations on the expression of *ftz*, *cad* and *Kr*, as visualized by antibody stainings: *a*, *ftz* expression in wild-type at blastoderm stage³; *b-e*, effect of increasing strength of the gain-of-function effect on *ftz* expression (*tor*^{Y9}/*tor*⁺, *tor*^{Y9}/*tor*⁺, *tor*^{RL3}/*tor*^{RL3}, *tor*⁴⁰²¹/*tor*⁺, respectively). *ftz* expression retracts from the poles and finally fades away, so the seventh stripe is the least sensitive; it is not present in most embryos of *tor*^{Y9}/*tor*⁺ (not shown). *f*, In a lack-of-function mutant embryo (*tor*^{PM}/*tor*^{HH}), the seventh stripe is missing, and the remaining stripes are expanded towards the poles, especially towards the posterior pole⁹; *g-h*, *cad* expression. In wildtype (*g*), at the extended germ band stage, the anal region and the internalized tissue of posterior midgut and Malpighian tubules express *cad*¹¹. In an embryo of a strong *torso* gain-of-function phenotype (*tor*⁴⁰²¹/*tor*^{Y9}) at a comparable stage, the amount of *cad*-expressing tissue is grossly enlarged (optical sections using Nomarski optics). *i-j*, Expression of *Kr*. The large central domain in wildtype¹³ (*i*, arrow) is missing in a *tor*^{RL3}/*tor*^{RL3} embryo (29 °C). The more weakly expressed anterior and posterior *Kr* domains, which might overlap *tll* expression in wildtype, are still present in this mutant condition (*j*, arrowheads). The antibody stainings were performed according to the method described in ref. 11.

Lack- and gain-of-function *tor* alleles also behave in a complementary way in their impact on the blastoderm fate map. In *tor*⁻ embryos, the anlagen of the segmented regions of thorax and abdomen are expanded towards the poles^{2,9}, so the terminal anlagen are replaced by more central anlagen. This can be visualized by the expression pattern of the pair-rule gene *ftz* (Fig. 2*f*). In contrast, in gain-of-function mutant embryos, *ftz* expression becomes restricted to the centre of the embryo (Fig. 2*a-e*), and in strongly mutant embryos *ftz* expression is abolished. The increasing loss of *ftz* stripes correlates well with the defects observed in the cuticle of the mature embryos. The expression of another pair-rule gene, *even-skipped* (*eve*)¹⁰, is similarly affected (M. Frasch, personal communication). The reduction of the segmented anlagen in *tor* gain-of-function embryos, associated with an enlargement of the telson anlagen, is most obvious in the expression pattern of the *caudal* (*cad*) gene¹¹. In wild-type embryos after germband retraction, *cad* is expressed mainly in the posterior midgut, the Malpighian tubules, and in the anal region. In *tor* gain-of-function embryos exhibiting a strong phenotype, the amount of tissue that expresses *cad* is substantially enlarged to take up roughly the posterior two-thirds of the embryo (Fig. 2*g, h*). For the anterior terminal anlagen such an enlargement is not evident.

The gap gene *tailless* (*tll*) appears to be important in the suppression of the segmented region in the *tor* gain-of-function mutants. The *tll* phenotype is similar to that of *tor* and the other maternal terminal genes⁵ in that parts of the head skeleton as well as the cuticular portions of the telson are deleted (Fig. 3*c*). But unlike the (maternal) terminal genes, the most terminal structures (labrum and posterior midgut) are present. Embryos that are mutant for a *tor* gain-of-function allele, but lack *tll* zygotically, have a very similar phenotype to that of *tll* alone with deletion of the telson and parts of the head; however most

of the segments of thorax and abdomen that had been missing before are now present (Fig. 3*a, b*). Thus, in the absence of *tll*, the *tor* gain-of-function effect is suppressed, and segmentation is restored.

The similarity of the lack-of-function phenotypes of *tor* and *tll* suggests that both genes act in a common process to establish the terminal anlagen. As a zygotic gene, *tll* probably functions

Table 1 Rescue of *torso* mutants by cytoplasmic transplantations

Maternal genotype of donor	Position in donor (% egg length)	Developmental stage of donor embryos	Recipient <i>torso</i> embryos	
			Developed embryos (N)	Cases with telson structures formed
Wildtype	0-15	stage 2	113	24 (50)
Wildtype	40-60	stage 2	114	36 (60)
<i>tor</i> ^{PM} / <i>tor</i> ^{HH}	0-15	stage 2	182	0 (4)
<i>tor</i> ⁴⁰²¹ / <i>tor</i> ^{Y9}	40-60	stage 4	113	(42)
Wildtype	40-60	stage 4	62	(0)

The *torso* lack-of-function phenotype of embryos derived from *tor*^{PM}/*tor*^{HH} females can be rescued by the transplantation of wild-type cytoplasm. Cytoplasm was taken from posterior positions (0-15% egg length) and middle positions (40-60% egg length) of early cleavage stage (stage 2) donor embryos (before pole cell formation), and deposited near the posterior pole of recipient embryos of the same age. We scored for the rescue of telson structures by formation of spiracles. A less stringent criterion is the presence of filzkörper material (numbers given in brackets), which is occasionally also found in control embryos. For a detailed description of the method see ref. 19. The rescuing capacities of cytoplasm from posterior and from middle positions in wild-type embryos (Oregon R.) are similar. In contrast, cytoplasm from *torso*⁻ (*tor*^{PM}/*tor*^{HH}) embryos does not induce telson structures. Rescue activity ceases in syncytial blastoderm (stage 4) wild-type donors, whereas it persists in embryos mutant for *torso* gain-of-function alleles (in this experiment cytoplasm was derived from the dorsal cortical region, and the recipients were at stage 4). The anterior defects can also be rescued, if donor cytoplasm from anterior or middle positions is deposited near the anterior pole (data not shown).

downstream of the maternally required *tor* in the regulatory pathway. This hierarchy is confirmed by the epistasis of the *tll* phenotype in the double mutant. We would expect *tll*, like other gap genes, to be expressed at the blastoderm stage in those anlagen of the wild-type embryo that are deleted in *tll* mutant embryos (namely in the terminal regions). To explain the dependence of the *tor* gain-of-function effect on *tll* activity, we propose that in *tor* gain-of-function mutants, the *tll* gene product is ectopically present at middle regions of the embryo also. The ectopic *tll* product then suppresses other zygotic segmentation genes, resulting in a failure to form segments. The retraction of *ftz* expression from the poles, as seen in the phenotypic series (Fig. 2b-e), might thus reflect the concomitant expansion of *tll* activity from the poles to the centre, with increasing gain-of-function effect. Negative regulation of pair-rule genes at terminal positions could be one of the functions of *tll* in wildtype¹².

In addition to the pair-rule genes, the expression of at least one gap gene, *Krüppel* (*Kr*), is affected in *tor* gain-of-function embryos. In wildtype at the blastoderm stage, *Kr* is expressed in its chief domain in the centre of the egg, and in two additional stripes of weaker intensity¹³ (at 80% egglength, and at the posterior pole). In *tor* gain-of-function embryos of even intermediate phenotypic strength (*tor*^{RL3}/*tor*^{RL3}), the central domain (which is required for the segmentation function of *Kr*) is deleted, whereas the anterior and the posterior stripes persist (Fig. 2i, j). This is consistent both with the negative regulation of the central domain of *Kr* by terminal factors (ref. 14, and Ulrike Gaul, personal communication) and with the concept that gap genes interact negatively with each other^{15,16}. Part of the distortion in the pair-rule gene expression therefore might be achieved in an indirect manner.

The results from the double mutant of *tor* gain-of-function alleles and *tll* indicate that *tll* expression is normally restricted to the poles, and that one of the functions of *tor* is to activate the expression of *tll* in a spatially restricted manner. Using cytoplasmic transplantation, we addressed the question of whether the *tor* gene product itself is restricted to the terminal positions in wild-type embryos. When cytoplasm of wild-type embryos at the early cleavage stage is transplanted into *tor*⁻ recipients, phenotypic rescue is observed in a substantial proportion of the recipient embryos. Telson structures such as spiracles and tuft (which are never present in uninjected *tor* embryos) can be formed (Fig. 1f). But the rescuing activity does not appear to be localized, as cytoplasm taken from either middle or terminal positions has an equivalent capacity for rescue. Rescuing activity is not present in *tor*⁻ embryos, but is present and persists for longer in strong gain-of-function embryos: rescue activity is detectable up to the syncytial blastoderm stage in donors of the maternal genotype *tor*^{Y9/4021} whereas at this time in wild-type embryos almost no activity is found (Table 1). Thus, the rescuing activity is dependent on *tor* gene function, but our data do not suggest a localization of the *tor* gene product at these early stages of development.

Both the lack- and the gain-of-function phenotypes suggest that *tor* provides the cues required for correct spatial expression of terminal zygotic genes such as *tll*. Assuming that the *tor* gene product is indeed homogeneously distributed in the egg, we suggest two explanations for how it could exert its function. In one model, *tor* is an essential component of a cellular machinery that eventually generates a localized signal but which is initially not localized itself. In the gain-of-function mutants the signal is produced, but the localization process is defective. In a second model, *tor* transmits a pre-existing spatial signal, which might be provided by one of the other terminal genes, to *tll* and possibly to other terminal target genes. This transmission could involve an activating modification of the *tor* product. A constitutively active mutant gene product would then be independent of the spatial signal, and result in the gain-of-function phenotype. A similar model has been proposed for the system of maternal genes that establishes the dorso-ventral polarity in the *Drosophila*

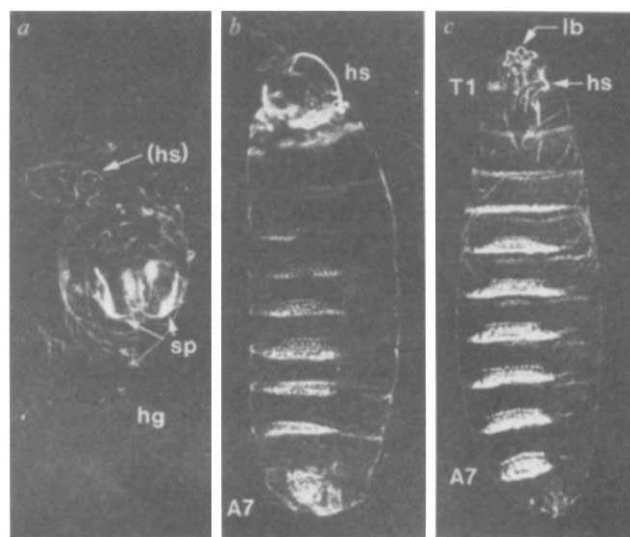


Fig. 3 The phenotype of the double mutant of a *torso* gain-of-function allele and *tll*. 75% of the eggs laid by females of the genotype *tor*⁴⁰²¹/*tor*⁺; *tll*¹/*tll*⁺, crossed to *tll*¹/TM3 males, develop the phenotype shown in a; the other 25% develop the one shown in b. a, Embryo of the 75% class. This embryo exhibits the maternal effect of the dominant *torso* allele, but not the zygotic one of *tll*. b, Double mutant embryo. It is almost identical to that of *tll* alone; the segmentation defects in *tor*⁴⁰²¹/*tor*⁺ (see b) are largely neutralized. Head and posterior abdominal defects are slightly stronger. The remaining defects cannot be explained by the fact that *tll*¹ might not be amorphic⁵, because they are also observed in double mutants using the deficiencies *tll*⁸ and *tll*⁶. c, Phenotype of a *tll*¹/*tll*¹ embryo; in contrast to *tor* (see Fig. 1e), the labrum (lb) is present (as well as the posterior midgut, which is not visible here).

embryo in relation to the action of the *Toll* gene. Like *tor*, *Toll* has dominant alleles which exhibit a phenotype opposite (ventralized embryos) to that of the lack-of-function alleles (dorsalized embryos)¹⁷. As with *tor*, the *Toll* rescue activity does not seem to be localized¹⁸. Molecular analysis of the *torso* gene and its mutant alleles will provide further insights into its mechanism of action, and possible analogies with the dorso-ventral pattern forming system.

We thank T. Schüpbach for the gift of the *tor*^{RL3} stock, U. Mayer and R. Lehmann for their contributions in the isolation of the *tor*^{Y9} allele, J. Lengyel for the *tll* deficiencies, S. Carroll, G. Struhl and U. Gaul for providing antibodies, H. G. Frohnhofer and G. Jürgens for discussion, our colleagues in Tübingen for comments on the manuscript and R. Grömke-Lutz for photographs.

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Review Articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance but must not exceed six pages of *Nature*.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

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All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

Colour Artwork. A charge of £500 a page is made for 4-colour figures. Inability to meet these costs will not prevent the publication of essential colour figures if the circumstances are explained.

Figure legends must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963–65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

A guide to ion-selective electrodes

D.C. Hixon

Electrodes are now on the market for the measurement of nearly two dozen different ions, some down to attomolar concentrations. Tips on their use are outlined below.

ION-SELECTIVE electrodes have been used in practical laboratory applications for over 25 years, and can be categorized into four basic types: glass, solid-state, liquid membrane, and gas-sensing. Glass electrodes only measure sodium ion and pH, while solid-state electrodes use a pellet based on an inorganic salt to determine the concentration of a wide variety of anions and cations in solution. Liquid membrane electrodes are based on liquid ion exchangers which contain large organic molecules, and are useful for measuring biologically significant ions. Gas-sensing electrodes measure dissolved gases in solution through a sensing element separated from the sample by a gas-permeable membrane.

Most ion-selective electrodes are responsive over wide concentration ranges, from 10^{-6} M to saturated solutions.

The table lists commonly available electrodes and their concentration ranges, but careful measurement may allow analysis at even lower ion concentration levels. Metal ion electrodes are especially sensitive: cupric ion electrodes have been shown to have linear responses at the attomolar level (A. Avdeef, J. Zabronsky and H. Stuting, Syracuse University, New York, personal communication).

The ability of an electrode to measure ion concentration is dependent upon a millivolt potential developed in solution — not the light reflected or absorbed by a sample — so the sample's colour or turbidity has no effect on analysis. Ion-selective electrodes are also rugged: with the exception of glass electrodes, most are available in unbreakable bodies made of epoxy or other durable inert materials.

Rapid response

The response time of ion-selective electrodes is usually less than two minutes per sample, but many achieve a 95 per cent or better response in less than one minute, depending on the ion concentration level and the sample conditions. Most ion measurement techniques are non-destructive, allowing *in situ* measurements to be performed. Continuous real-time measurements are also possible, because ion-selective electrodes produce a stable millivolt potential which can be monitored over time.

Ion-selective electrodes are relatively low in cost: a complete setup including instrument, electrodes and reagents can be purchased for less than \$1,000 (US). In comparison, other instruments which are

used to measure ion concentrations, such as atomic absorption units or ion chromatographs, cost more than \$10,000 (US). Operation costs are minimal because the

ease of use of electrodes eliminates the need for highly skilled technicians. The basic calibration and measurement techniques may be adapted to the production

Various ion-selective electrodes

Electrode	Type	Concentration range	Applications
Ammonia (NH ₃) [ammonium (NH ₄ ⁺)]	Gas-sensing	1.0 to 5×10^{-7} M 17,000 to 0.01 p.p.m.	Air, water, wastewater, soil, wine
Bromide (Br ⁻)	Solid-state	1.0 to 5×10^{-6} M 79,900 to 0.40 p.p.m.	Soil, plant tissue, wine, aqueous samples
Cadmium (Cd ²⁺)	Solid-state	10^{-1} to 10^{-7} M 11,200 to 0.01 p.p.m.	Plating baths, aqueous samples
Calcium (Ca ²⁺)	Liquid membrane	1.0 to 5×10^{-7} M 40,100 to 0.02 p.p.m.	Beer, milk, soil, fertilizer, water, feed
Carbon dioxide (CO ₂) [carbonate (CO ₃ ²⁻)]	Gas-sensing	10^{-2} to 10^{-1} M 440 to 4.4 p.p.m.	Bacterial cultures, beverages, wine, alkaline plating solutions
Chloride (Cl ⁻)	Solid-state	1.0 to 5×10^{-5} M 35,500 to 1.8 p.p.m.	Food, beverages, plant tissue, plating baths, sweat, sea water, brine, water, wastewater, soil
Chlorine (Cl ₂)	Solid-state	3×10^{-4} to 10^{-7} M 20 to 0.01 p.p.m.	Drinking water, wastewater
Cupric (Cu ²⁺)	Solid-state	0.1 to 10^{-8} M 6,350 to 6.4×10^{-4} p.p.m.	Plating baths, water
Cyanide (CN ⁻)	Solid-state	10^{-2} to 8×10^{-6} M 260 to 0.2 p.p.m.	Biological materials, plant tissue, plating baths, water, wastewater, brine
Fluoride (F ⁻)	Solid-state	Saturated to 10^6 M saturated to 0.02 p.p.m.	Drinking water, toothpaste, bone, dental plaque, geological samples, acids, biological fluids
Fluoroborate (BF ₄ ⁻)	Liquid membrane	1.0 to 7×10^{-6} M 86,800 to 0.61 p.p.m.	Plating baths
Iodide (I ⁻)	Solid-state	1.0 to 5×10^{-8} M 127,000 to 5×10^{-3} p.p.m.	Milk, feed, plants, water, food
Lead (Pb ²⁺)	Solid-state	0.1 to 10^{-6} M 20,700 to 0.2 p.p.m.	Water, organic compounds, paint
Nitrate (NO ₃ ⁻)	Liquid membrane	1.0 to 7×10^{-6} M 14,000 to 0.1 p.p.m. as N	Water, plant tissue, soil, fertilizer, food
Nitrogen oxide (NO _x) [nitrite (NO ₂ ⁻)]	Gas-sensing	5×10^{-3} to 4×10^{-6} M 230 to 0.18 p.p.m.	Air, stack gases, soil, water, food
Oxygen (O ₂)	Gas-sensing	0–14 p.p.m. (nominal)	Biological oxygen demand (BOD), water
Perchlorate (ClO ₄ ⁻)	Liquid membrane	1.0 to 7×10^{-6} M 99,500 to 0.7 p.p.m.	Explosives, solid propellants, aqueous samples
Potassium (K ⁺)	Liquid membrane	1.0 to 10^{-6} M 39,000 to 0.04 p.p.m.	Soils, fertilizer, wines, biological samples
Silver/sulphide (Ag ⁺ /S ²⁻)	Solid-state	Ag ⁺ : 1.0 to 10^{-7} M 107,900 to 0.01 p.p.m. S ²⁻ : 1.0 to 10^{-7} M 32,100 to 0.003 p.p.m.	Ag ⁺ : plating baths S ²⁻ : water, sediments, plant materials, proteins
Sodium (Na ⁺)	Glass	Saturated to 10^{-6} M saturated to 0.02 p.p.m.	Food, beverages, soil, water glass, plant tissue
Surfactant	Liquid membrane	Minimum pure SLS titratable: 10^{-3} M maximum with 0.05M hyamine: 0.05M	Soaps, detergents, toothpaste, household products
Thiocyanate (SCN ⁻)	Solid-state	1.0 to 5×10^{-6} M 58,100 to 0.29 p.p.m.	Aqueous samples
Water hardness (X ²⁺)	Liquid membrane	1 to 6×10^{-6} M	Water

Data in table from K. Cammann in *Chemical Laboratory Practice: Working with Ion-Selective Electrodes* 2nd edn, 163–170 (Springer, New York, 1979).

line, quality control laboratory, water and wastewater plant, as well as to the research laboratory.

The high selectivity of ion-selective electrodes may prove to be both a benefit and a hindrance. When the concentration of an interfering ion greatly exceeds that of the ion to be measured, the electrode's performance can be adversely affected.

In many cases, interference suppressors may be added to sample solutions to eliminate interfering ions. In addition, some samples may contain ions or compounds which can contaminate the electrodes, requiring frequent cleaning or treatment during use. Most manufacturers publish information listing which species contaminate or impair the operation of their electrodes.

Automation

Ion-selective electrodes may be used for direct calibration of a given ion, incremental measurement techniques such as known addition, or titration analysis. Direct calibration allows a large number of samples to be analysed rapidly. Traditionally, a calibration curve is prepared by plotting the millivolt values versus concentration on semilogarithmic graph paper, and the measurement of each sample is compared to the graph for interpretation. Instrumentation is now commercially available which does this automatically for the analyst.

Incremental measurement techniques, where a known amount of a specific substance is added to the sample, can enhance the specificity of ion-selective electrode measurements. Titrating the sample can expand the number of species measurable by electrode. The advent of sophisticated automatic titrators allows fast, rapid analysis, limited sample handling, and data calculation with a minimum of effort on the part of the analyst.

Recently, improvements in the area of ion-selective electrodes have focused on both the instrumentation and the electrodes themselves. Sophisticated electronics and software have expanded the capabilities of the traditional millivolt meter to include techniques such as double known addition and automated gran analysis. Technical improvements in electrodes include temperature insensitivity and improved reference electrode junctions.

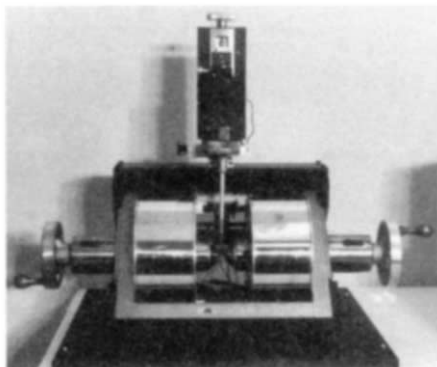
New sensing electrodes are continually being developed and made available commercially. In general, ion-selective electrodes are a practical measuring tool for the chemist, and will continue to be so in the future □

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Measures for measurement

Electronic instruments for the measurement of a wide variety of variables are featured this week, including a windvane and a magnetometer for studying superconductors.

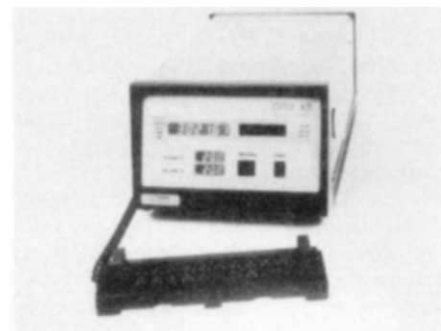
PRINCETON Measurements Corporation has an **alternating force magnetometer** that it says is many times more sensitive — and considerably smaller and faster — than vibrating-sample magnetometers and SQUID-type instruments for the characterization of magnetic and superconducting materials (*Reader Service No. 101*). The \$63,000 (US) model 2900 MicroMag makes use of the alternating gradient force technique, in conjunction with a piezoelectric transducer, to measure the magnetic properties of thin films and powders, and to determine the trace amounts of ferromagnetic impurities. It uses a compact 2-inch laboratory electro-



MicroMag, a timely entry into the 'superconductor market'.

magnet and a small 1.5 kW power supply, allowing it to fit easily onto a laboratory bench. MicroMag is designed to be sensitive down to 10^{-8} e.m.u., and has a measurement speed of 50 ms per point. The software is written in BASIC, and provides automatic procedures to assist the operator in positioning the sample, tuning for resonance, and determining various sample parameters, such as coercivity or retentivity, from the raw measurement data.

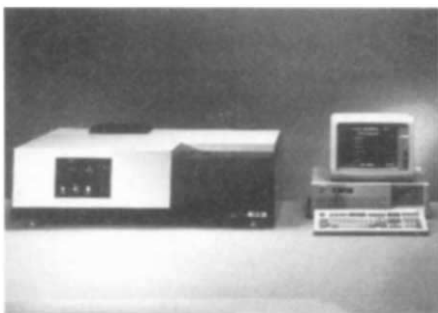
The model DMA48 **digital density meter** from Paar Scientific Ltd is a stand-alone, microprocessor-controlled instrument for the measurement of densities, the specific gravities of liquids and gases, and the concentrations of solutions (*Reader Service No. 102*). Paar Scientific says the DMA48 has an accuracy of $\pm 1 \times 10^{-4}$ g cm $^{-3}$, and is equipped with a solid-state thermostat with a temperature range of -10°C to $+70^\circ\text{C}$. Calibration is carried out by the system's computer, which will also store and automatically check the constants of the oscillator, and the temperature and date. The values of more than 20 different temperatures and remote cells can be stored, as well as



Paar's DMA48 density meter.

density values for air and water over the temperature range $0-60^\circ\text{C}$. The DMA48 is equipped with connections for a filling/rinsing device, automatic sample changer, metal cell, analogue output, remote control and documentation via a personal computer.

Jobin Yvon has recently announced the introduction of its model CD-6 **dichrograph** for the measurement of the circular dichroism of drugs, proteins and DNA in the biotechnology and pharmaceutical industries (*Reader Service No. 103*). Jobin Yvon says the CD-6 takes up less bench space, has faster warm-up time and is more stable and easier to use than its predecessors. When purged, the company says the CD-6 can make measurements down to 180 nm with only a small flow of nitrogen. The dichrograph is equipped with an IBM/PC-AT, and the multi-tasking software, written in Turbo Pascal, performs data acquisition and manipula-



Circular dichroism and data manipulation: the CD-6.

tion as well as spectral calibration. Optional equipment for the CD-6 dichrograph includes accessories for optical rotary dichroism, linear dichroism, magnetic circular dichroism, flow-through cells and kinetics packages.

Firecracker is the name of a new **bio-chemical sensing system** from Cambridge

Consultants Ltd that can be used to automate the technique of monitoring antibody-antigen reactions (*Reader Service No. 104*). The company says the Firecracker can follow the time profile of simultaneous antigenic reactions without operator supervision. The Firecracker works by linking the antibody to a fluorescent material and immobilizing it on the surface of a microscope slide that is optically coupled to a prism. Light from a laser is guided by the prism into the slide in such a way that total internal reflection occurs



Antibody-antigen interactions are pinned down in the Firecracker from Cambridge Consultants.

on the antibody surface, creating an evanescent wave which excites the fluorescence, allowing the course of the antibody-antigen reaction to be followed. The Firecracker has applications in medical screening, the monitoring of environmental pollution, and the detection of drugs of abuse.

Environs analysis

Heto Lab Equipment has added a new Hetotherm **thermostat** dubbed the model DBT623 to its line of laboratory thermostats (*Reader Service No. 105*). Heto says the model DBT623 is a multi-purpose digital thermostat designed for baths of a minimum depth of 150 mm, that covers a temperature range of -50°C to $+200^{\circ}\text{C}$. All control buttons for the model DBT623 are accessible from the front, and it has a large bright display to make temperature reading easier. The thermostat has a comprehensive safety system which sets off an alarm if the unit overheats, if the liquid level drops too low, or if the motor burns out. It is provided with a connector for the remote control of start/stop, alarm output, actual temperature readout, differential temperature readout, and set point adjustment.

For the measurement of **windspeed and direction**, Vector Instruments offers an optoelectronic Porton anemometer and windvane, and its D600 series of displays (*Reader Service No. 106*). The model A100 anemometer and the model W200 fixed north reference windvane are constructed from anodized aluminium alloys, stainless steel and weather-resistant plastics. Vector Instruments says they can withstand permanent exposure to any type of weather, and winds of up to 170

m.p.h. The model D600 range of control and display units provide recorder and logger outputs and come with a 12-V power supply. The anemometer and windvane can be adapted with optional anti-icing heaters and modified neoprene cables for use at low temperatures.

Spectroscopy specifics

Autoscribe Ltd now offers a three-part **computer tutorial series** for teaching the basic principles of spectral interpretation (*Reader Service No. 107*). The infrared spectroscopy tutorial divides up the infrared spectrum into six specific areas, and discusses the vibrational modes responsible for the absorptions in each area, teaching the student to examine them for the presence or absence of absorption bands indicative of particular functional groups. The nuclear magnetic resonance (NMR) spectroscopy program introduces all of the components necessary to interpret NMR spectra, including solvents and reference compounds, proton equivalence, the calculation of chemical shifts of protons, and multiplicities and integration. The carbon-13 magnetic resonance spectroscopy (CMR) tutorial defines terms useful in CMR spectroscopy, and allows students to examine characteristic chemical shifts in carbon-13 spectra. The tutorial programs are available for both the Apple II computer and the IBM PC.

Jeol has a new 90 MHz **nuclear magnetic resonance (NMR) spectrometer**, the EX-90, for routine and research measurements (*Reader Service No. 108*). The EX-90 features advanced digital quadrature detection for high sensitivity and a 32-bit data word length computer for fast computation. It is provided with software for multi-tasking with DEC-net and Ethernet electronic networks, while processing the NMR spectra in parallel. Omni probes for multinuclear analysis



A 32-bit computer augments the Jeol NMR spectrometer.

and a pulse programmer for two-dimensional NMR are also included. The operator can obtain multiple displays of the main spectrum and expansions on one screen, and a help function is also included.

The Finnigan **MAT ion trap mass spectrometer** is now available with selec-

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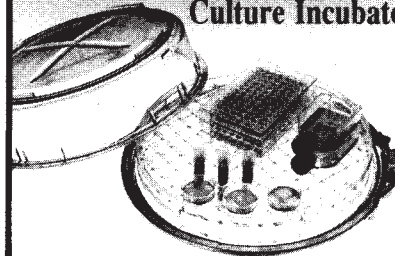
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tive mass storage, for the analysis of difficult compounds which occur at very low levels, such as pesticide residues in food (*Reader Service No. 109*). The company says the instrument's high sensitivity also makes it amenable to the study of complex ion/molecule interactions, investigations in ion physics, and the exploration of sophisticated analytical chemistry problems. Selective mass storage provides increased sensitivity by improving the efficiency of parent ion selection in the

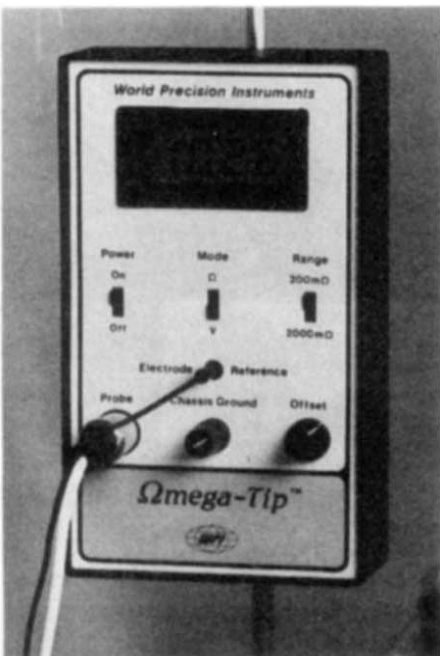


IBM-compatibility from Finnegan MAT.

MS/MS analysis. All instrument parameters are software-programmable via an IBM PS/2 or compatible computer. Both electron ionization and chemical ionization modes of operation can be selected without a change in hardware.

Electrode essentials

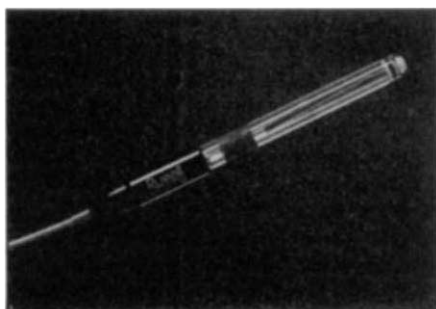
The Omega-Tip **millivolt and megohm meter** from World Precision Instruments can be used to measure the resistance of electrolyte-filled micropipette electrodes in intracellular experiments (*Reader Service No. 110*). Created specifically to measure micropipette electrode impedance, the \$695 (US) Omega-Tip features a resistance-measuring circuit which is



The WPI Omega-Tip, at under \$700.

unaffected by electrode offset and tip junction potentials, says WPI. The company says resistances of up to 2,000 megohms can be measured and displayed on the Omega-Tip's digital LCD. A gold-plated miniature probe lets the user monitor microelectrode resistance while beveling pulled-glass microcapillary electrodes. The battery-operated Omega-Tip can also be used to measure d.c. electrode tip potential up to 2,000 mV.

Russell pH Ltd has developed a flat-head electrode for the direct pH measurement of electrophoresis gels (*Reader Service No. 111*). The Russell model CFWL/30/6.6/+b **electrophoresis electrode** provides an alternative method for measuring pH at the isoelectric point,



Gel pH can be measured directly with the new Russell electrode.

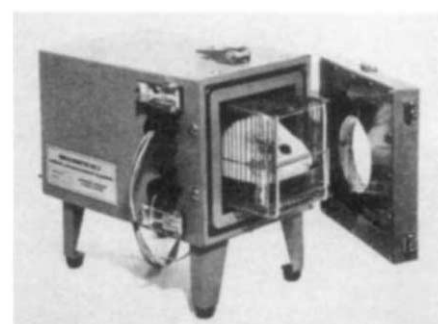
directly on the gel. The combination electrode consists of a 4-mm flat-head pH sensing glass which is surrounded by an annular Teflon junction. The reference cell completes the measuring circuit by contact through the junction.

The fifth edition of Fisher Scientific's **electrode handbook** is just off the presses (*Reader Service No. 112*). The handbook is a comprehensive manual on pH and ion-selective electrodes, and is designed to serve as a reference for industrial, biomedical, governmental, educational and research laboratories. Included in the handbook are sections on working theory, electrode types, care of electrodes, troubleshooting, and pH and ion-selective instrumentation. The 30-page free handbook is pre-punched for binders, and features 60 colour actual-size illustrations of Fisher ion-selective, metallic, pH-indicating, combination-pH, and reference electrodes.

Calorimetry counts

A new **nutrition biocalorimeter** from Thermochemical Corporation can be used to measure the metabolic effects of diet, activity, medication, or stress in humans and in animals (*Reader Service No. 113*). Subjects can live in the calorimeter — available in various sizes — for as long as required. Air supply and temperature are controlled, and windows, doors, electrical and instrument connectors, and ports for the introduction of food and the removal

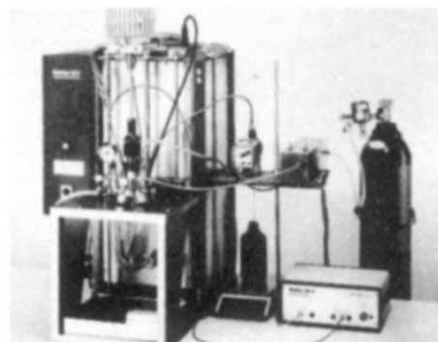
of wastes are available. The biocalorimeter measures all of the subject's heat outputs, including conductive, radiative and latent heat, in a d.c. linear format,



Man-size versions also available . . .

which can be recorded separately. The biocalorimeters can be made to order with capacities of 1 cubic inch to 1,000 cubic feet, at prices of between \$2,500 and \$500,000 (US).

MSE Scientific Instruments sells the Mettler RC1 **reaction calorimeter**, a medium-pressure reactor which can measure chemical processes requiring conditions of up to 12 bars of pressure (*Reader Service No. 114*). The jacketed reactor is manufactured from double-walled glass to withstand both heat and reactive chemicals while allowing the reaction to be viewed from outside. The model RC1 reactor can be used to determine the heat flow of chemical reactions and physical transformations in the laboratory, so that large-scale processes can be developed and optimized, and small quantities of product can be produced



The Mettler RC1 contains and monitors a chemical reaction.

efficiently, says the company. While the reaction is taking place, the temperature, pressure, pH and other conditions can be controlled from a computer, and additional reaction components can be added automatically to the reactor. The measured values are displayed on the computer monitor, and can be printed out on a printer-plotter. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.